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[Continued on next page]

(54) Title: NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

(57) Abstract: Disclosed herein are nucleic acid sequences that encode G-coupled protein-receptor related polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies, which immunospecifically-bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel human nucleic acids and proteins.

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## NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

### BACKGROUND OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides. More particularly, the invention relates to nucleic acids encoding novel G-protein coupled receptor (GPCR) polypeptides, as well as vectors, host cells, antibodies, and recombinant methods for  
5 producing these nucleic acids and polypeptides.

### SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of nucleic acid sequences encoding novel polypeptides. The novel nucleic acids and polypeptides are referred to herein as  
10 GPCR<sub>X</sub>, or GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, GPCR7, GPCR8, and GPCR9 nucleic acids and polypeptides. These nucleic acids and polypeptides, as well as derivatives, homologs, analogs and fragments thereof, will hereinafter be collectively designated as "GPCR<sub>X</sub>" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated GPCR<sub>X</sub> nucleic acid molecule  
15 encoding a GPCR<sub>X</sub> polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids disclosed in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82. In some embodiments, the GPCR<sub>X</sub> nucleic acid molecule will hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of a GPCR<sub>X</sub> nucleic acid  
20 sequence. The invention also includes an isolated nucleic acid that encodes a GPCR<sub>X</sub> polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide comprising the amino acid sequences of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83. The nucleic acid can be, for example, a genomic DNA fragment or a  
25 cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a GPCR<sub>X</sub> nucleic acid (*e.g.*, SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82) or a  
30 complement of said oligonucleotide.



Also included in the invention are substantially purified GPCR<sub>X</sub> polypeptides (SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83). In certain embodiments, the GPCR<sub>X</sub> polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human GPCR<sub>X</sub> polypeptide.

5       The invention also features antibodies that immunoselectively bind to GPCR<sub>X</sub> polypeptides, or fragments, homologs, analogs or derivatives thereof.

      In another aspect, the invention includes pharmaceutical compositions that include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a GPCR<sub>X</sub> nucleic acid, a GPCR<sub>X</sub>  
0       polypeptide, or an antibody specific for a GPCR<sub>X</sub> polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

      In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a GPCR<sub>X</sub> nucleic acid, under conditions allowing for expression  
5       of the GPCR<sub>X</sub> polypeptide encoded by the DNA. If desired, the GPCR<sub>X</sub> polypeptide can then be recovered.

      In another aspect, the invention includes a method of detecting the presence of a GPCR<sub>X</sub> polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex  
10       between the polypeptide and the compound. The complex is detected, if present, thereby identifying the GPCR<sub>X</sub> polypeptide within the sample.

      The invention also includes methods to identify specific cell or tissue types based on their expression of a GPCR<sub>X</sub>.

      Also included in the invention is a method of detecting the presence of a GPCR<sub>X</sub>  
15       nucleic acid molecule in a sample by contacting the sample with a GPCR<sub>X</sub> nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to a GPCR<sub>X</sub> nucleic acid molecule in the sample.

      In a further aspect, the invention provides a method for modulating the activity of a GPCR<sub>X</sub> polypeptide by contacting a cell sample that includes the GPCR<sub>X</sub> polypeptide with a  
30       compound that binds to the GPCR<sub>X</sub> polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, or other disorders related to cell signal processing and metabolic pathway modulation. The therapeutic can be, *e.g.*, a GPCR<sub>X</sub> nucleic acid, a GPCR<sub>X</sub> polypeptide, or a GPCR<sub>X</sub>-specific antibody, or biologically-active derivatives or fragments thereof.

For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: developmental diseases, MHCII and III diseases (immune diseases), taste and scent detectability Disorders, Burkitt's lymphoma, corticoneurogenic disease, signal transduction pathway disorders, Retinal diseases including those involving photoreception, Cell growth rate disorders; cell shape disorders, feeding disorders; control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidoluysian atrophy (DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding GPCR<sub>X</sub> may be useful in gene therapy, and GPCR<sub>X</sub> may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia,

asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders or other disorders related to cell signal processing and metabolic pathway modulation. The method includes contacting a test compound with a GPCR<sub>X</sub> polypeptide and determining if the test compound binds to said GPCR<sub>X</sub> polypeptide. Binding of the test compound to the GPCR<sub>X</sub> polypeptide indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders or other disorders related to cell signal processing and metabolic pathway modulation by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a GPCR<sub>X</sub> nucleic acid. Expression or activity of GPCR<sub>X</sub> polypeptide is then measured in the test animal, as is expression or activity of the protein in a control animal which recombinantly-expresses GPCR<sub>X</sub> polypeptide and is not at increased risk for the disorder or syndrome. Next, the expression of GPCR<sub>X</sub> polypeptide in both the test animal and the control animal is compared. A change in the activity of GPCR<sub>X</sub> polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

In yet another aspect, the invention includes a method for determining the presence of or predisposition to a disease associated with altered levels of a GPCR<sub>X</sub> polypeptide, a GPCR<sub>X</sub> nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes measuring the amount of the GPCR<sub>X</sub> polypeptide in a test sample from the subject and  
5 comparing the amount of the polypeptide in the test sample to the amount of the GPCR<sub>X</sub> polypeptide present in a control sample. An alteration in the level of the GPCR<sub>X</sub> polypeptide in the test sample as compared to the control sample indicates the presence of or predisposition to a disease in the subject. Preferably, the predisposition includes, *e.g.*, diabetes, metabolic disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders  
10 associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. Also, the expression levels of the new polypeptides of the invention can be used in a method to screen for various cancers as well as to determine the stage of cancers.

15 In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject a GPCR<sub>X</sub> polypeptide, a GPCR<sub>X</sub> nucleic acid, or a GPCR<sub>X</sub>-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes, *e.g.*, diabetes, metabolic  
20 disturbances associated with obesity, the metabolic syndrome X, anorexia, wasting disorders associated with chronic diseases, metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

In yet another aspect, the invention can be used in a method to identity the cellular  
25 receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention  
30 belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the

present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

5

## DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode novel polypeptides. The novel nucleic acids and their encoded polypeptides are referred to individually as GPCR1, GPCR2, GPCR3, GPCR4, GPCR5, GPCR6, GPCR7, GPCR8, and GPCR9. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "GPCRX".

The novel GPCRX nucleic acids of the invention include the nucleic acids whose sequences are provided in Tables 1A, 1D, 1E, 2A, 3A, 4A, 4F, 4J, 5A, 5D, 5F, 6A, 6D, 7A, 7F, 7I, 8A, 8D, 8F, 8H, 8J, 9A, 9D, and 9F, inclusive ("Tables 1A - 9F"), or a fragment, derivative, analog or homolog thereof. The novel GPCRX proteins of the invention include the protein fragments whose sequences are provided in Tables 1B, 1F, 2B, 3B, 4B, 4G, 5B, 5E, 5G, 6B, 6E, 7C, 7G, 7J, 8B, 8E, 8G, 8I, 8K, 9B, 9E, and 9G, inclusive ("Tables 1B - 9G"). The individual GPCRX nucleic acids and proteins are described below. Within the scope of this invention is a method of using these nucleic acids and peptides in the treatment or prevention of a disorder related to cell signaling or metabolic pathway modulation.

G-Protein Coupled Receptor proteins (GPCRs) have been identified as a large family of G protein-coupled receptors in a number of species. These receptors share a seven transmembrane domain structure with many neurotransmitter and hormone receptors, and are likely to underlie the recognition and G-protein-mediated transduction of various signals. Human GPCR generally do not contain introns and belong to four different gene subfamilies, displaying great sequence variability. These genes are dominantly expressed in olfactory epithelium. See, *e.g.*, Ben-Arie et al., Hum. Mol. Genet. 1994 3:229-235; and, Online Mendelian Inheritance in Man (OMIM) entry # 164342 (<http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?>).

The olfactory receptor (OR) gene family constitutes one of the largest GPCR multigene families and is distributed among many chromosomal sites in the human genome. See Rouquier et al., Hum. Mol. Genet. 7(9):1337-45 (1998); Malnic et al., Cell 96:713-23 (1999). Olfactory receptors constitute the largest family among G protein-coupled receptors, with up to 1000 members expected. See Vanderhaeghen et al., Genomics 39(3):239-46

(1997); Xie et al., *Mamm. Genome* 11(12):1070-78 (2000); Issel-Tarver et al., *Proc. Natl. Acad. Sci. USA* 93(20):10897-902 (1996). The recognition of odorants by olfactory receptors is the first stage in odor discrimination. See Krautwurst et al., *Cell* 95(7):917-26 (1998); Buck et al., *Cell* 65(1):175-87 (1991). Many ORs share some characteristic sequence motifs and have a central variable region corresponding to a putative ligand binding site. See Issel-Tarver et al., *Proc. Natl. Acad. Sci. USA* 93:10897-902 (1996).

Other examples of seven membrane spanning proteins that are related to GPCRs are chemoreceptors. See Thomas et al., *Gene* 178(1-2):1-5 (1996). Chemoreceptors have been identified in taste, olfactory, and male reproductive tissues. See *id.*; Walensky et al., *J. Biol. Chem.* 273(16):9378-87 (1998); Parmentier et al., *Nature* 355(6359):453-55 (1992); Asai et al., *Biochem. Biophys. Res. Commun.* 221(2):240-47 (1996).

### GPCR1

GPCR1 includes a family of three novel G-protein coupled receptor ("GPCR") proteins disclosed below. The disclosed proteins have been named GPCR1a, GPCR1b, and GPCR1c and are related to olfactory receptors.

#### GPCR1a

A disclosed GPCR1a nucleic acid of 1050 nucleotides is shown in Table 1A. The disclosed GPCR1a open reading frame ("ORF") begins at the ATG initiation codon at nucleotides 6-8, shown in bold in Table 1A. The encoded polypeptide is alternatively referred to herein as GPCR1a or as rp11-507n20\_A. The disclosed GPCR1a ORF terminates at a TGA codon at nucleotides 1044-1046. As shown in Table 1A, putative untranslated regions 5' to the start codon and 3' to the stop codon are underlined, and the start and stop codons are in bold letters.

**Table 1A. GPCR1a nucleotide sequence (SEQ ID NO:1).**

```

CGCCATGTACAACGGGTCGTGCTGCCGCATCGAGGGGACACCATCTCCAGGTGATGCCGCCGCTGCTCATTGTG
GCCTTTGTGCTGGGCGCACTAGGCAATGGGGTCGCCCTGTGTGGTTTCTGCTTCCACATGAAGACCTGGAAGCCAG
CACTGTTTACCTTTTCAATTTGGCCGTGGCTGATTTCTCCTTATGATCTGCCTGCCTTTTCGGACAGACTATTACC
TCAGACGTAGACACTGGGCTTTTGGGACATTCCCTGCCGAGTGGGGCTTTCACGTTGGCCATGAACAGGGCCGGG
AGCATCGTGTTCCTTACGTTGGTGGCTGCGGACAGGTATTTCAAAGTGGTCCACCCCCACCACGGGTGAACACTAT
CTCCACCCGGGTGGCGGTGGCATCGTCTGCACCTGTGGGCCCTGGTCATCCTGGGAACAGTGTATCTTTTGTGG
AGAACCATCTCTCGGTGCAAGAGACGGCCGTCTCCTGTGAGAGCTTCATCATGGAGTCGGCCAATGGCTGGCATGAC
ATCATGTTCCAGCTGGAGTTCCTTATGCCCCCTCGGCATCATCTATTTTGTCTCCTTCAAGATTGTTTGGAGCCTGAG
GCGGAGGCAGCAGCTGGCCAGACAGGCTCGGATGAAGAAGCGACCCGGTTCATCATGGTGGTGGCAATTGTGTTCA
TCACATGCTACCTGCCAGCGTGTCTGTAGACTCTATTTCTCTGGACGGTGCCTCGAGTGCCTGCGATCCCTCT
GTCCATGGGGCCCTGCACATAACCCTCAGCTTCACCTACATGAACAGCATGCTGGATCCCTGGTGTATTATTTTC
AAGCCCTCCTTTCCCAATTCTACAACAAGCTCAAAATCTGCAGTCTGAAACCCAAGCAGCCAGGACACTCAAAA
CACAAAGGCCGAAGAGATGCCAATTTCGAACCTCGGTGCGAGAGTTGCATCAGTGTGGCAAATAGTTTCCAAAGC
CAGTCTGATGGGCAATGGGATCCCCACATTGTTGAGTGGCACTGAACAA

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A disclosed encoded GPCR1a protein has 346 amino acid residues, referred to as the GPCR1a protein. The GPCR1a protein was analyzed for signal peptide prediction and cellular localization. SignalP results predict that GPCR1a is cleaved between position 32 and 33 of SEQ ID NO:2, *i.e.*, in the amino acid sequence ALG-NG. Psort and Hydropathy profiles also predict that GPCR1 contains a signal peptide and is likely to be localized in the endoplasmic reticulum (Certainty 0.6850) or the plasma membrane (Certainty=0.6400). The disclosed GPCR1 polypeptide sequence with a molecular weight of 39294.8 Daltons is presented in Table 1B using the one-letter amino acid code.

**Table 1B. Encoded GPCR1a protein sequence (SEQ ID NO:2).**

```
MYNGSCCRIEGDTISQVMPPLLIIVAFVIGALGNGVALCGFCFHMKTWKPSTVYLFNLAVADFLLMICLPFRTDYLR
RRHWAFGDI PCRVGLFTLAMNRAGSIVFLTVVAADRYFKVVHPHAVNTISTRVAAGIVCTLWALVILGTVYLLLEN
HLCVQETAVSCSFIMESANGWHDIMFQLEFFMPLGIILFCSEKIVWSLRRRQQLARQARMKKATRFIMVVAIVFIT
CYLPSVSARLYFLWTVPSACDPSVHGALHITLSFTYMNSMLDPLVYFSSPSFPKFYNKLIKICSLKPKQPQGHSTQ
RPEEMPISNLGRRSCISVANSEFQSDGQWDFHIVEWH
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GPCR1a was initially identified on chromosome 12 with a TblastN analysis of a proprietary sequence file for a G-protein coupled receptor probe or homolog, which was run against the Genomic Daily Files made available by GenBank. A proprietary software program (GenScan™) was used to further predict the nucleic acid sequence and the selection of exons. The resulting sequences were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein.

A region of the GPCR1a nucleic acid sequence has 517 of 746 bases (69%) identical to a *Homo sapiens* GPCR mRNA (GENBANK-ID: HUMHM74|acc:D10923), with an E-value of  $1.9 \times 10^{-71}$ . In all BLAST alignments herein, the "E-value" or "Expect" value is a numeric indication of the probability that the aligned sequences could have achieved their similarity to the BLAST query sequence by chance alone, within the database that was searched. For example, the probability that the subject ("Sbjct") retrieved from the GPCR1a BLAST analysis, *e.g.*, the *Homo sapiens* GPCR, matched the Query GPCR1a sequence purely by chance is  $1.9 \times 10^{-71}$ .

A BLASTX search was performed against public protein databases. The full amino acid sequence of the protein of the invention was found to have 178 of 339 amino acid residues (52%) identical to, and 227 of 339 residues (66%) positive with, the 387 amino acid residue protein from *species* (ptnr: SWISSPROT-ACC:P49019).

The amino acid sequence of GPCR1a also had high homology to other proteins as shown in table 1C.

Table 1C. BLASTX alignments of GPCR1a

Sequences producing High-scoring Segment Pairs:			Smallest
	Reading Frame	High Score	Sum Prob. P(N)
ptnr:SWISSPROT-ACC:O00270 PROBABLE G PROTEIN-COUPLED R...	+3	447	2.6e-41
ptnr:TREMBLNEW-ACC:AAF26668 G PROTEIN COUPLED RECEPTOR...	+3	441	1.1e-40
ptnr:SWISSPROT-ACC:P34996 P2Y PURINOCEPTOR 1 (ATP RECE...	+3	357	8.8e-32
ptnr:SWISSPROT-ACC:P49652 P2Y PURINOCEPTOR 1 (ATP RECE...	+3	357	8.8e-32
patp:W94654 G-protein coupled receptor HM74A protein -	+3	887	4.7e-88
patp:Y90672 Human mutant G protein-coupled receptor HM	+3	883	1.2e-87
patp:Y90637 Human G protein-coupled receptor HM74 - Ho.	+3	882	1.6e-87
patp:W88460 Human 7-transmembrane receptor HEOAD54 - H.	+3	524	1.4e-49
patp:Y94339 Human cell surface receptor protein #6 - H.	+3	524	1.4e-49

**GPCR1b**

In the present invention, the target sequence identified previously, Accession Number  
 5 rp11-507n20\_A, was subjected to the exon linking process to confirm the sequence. PCR  
 primers were designed by starting at the most upstream sequence available, for the forward  
 primer, and at the most downstream sequence available for the reverse primer. In each case,  
 the sequence was examined, walking inward from the respective termini toward the coding  
 sequence, until a suitable sequence that is either unique or highly selective was encountered,  
 10 or, in the case of the reverse primer, until the stop codon was reached. Such primers were  
 designed based on in silico predictions for the full length cDNA, part (one or more exons) of  
 the DNA or protein sequence of the target sequence, or by translated homology of the  
 predicted exons to closely related human sequences sequences from other species. These  
 primers were then employed in PCR amplification based on the following pool of human  
 15 cDNAs: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain -  
 hippocampus, brain - substantia nigra, brain - thalamus, brain -whole, fetal brain, fetal kidney,  
 fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary  
 gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen,  
 stomach, testis, thyroid, trachea, uterus. Usually the resulting amplicons were gel purified,  
 20 cloned and sequenced to high redundancy. The resulting sequences from all clones were  
 assembled with themselves, with other fragments in CuraGen Corporation's database and with  
 public ESTs. Fragments and ESTs were included as components for an assembly when the  
 extent of their identity with another component of the assembly was at least 95% over 50 bp..  
 In addition, sequence traces were evaluated manually and edited for corrections if appropriate.  
 25 These procedures provide the sequence reported below, which is designated Accession  
 Number rp11-507n20\_A\_da1.

A disclosed GPCR1b (also referred to as rp11-507n20\_A\_da1) nucleic acid of 1050



nucleotides is shown in Table 1D. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 6-8 and ending with a TGA codon at nucleotides 1044-1046. A putative untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 1D, and the start and stop codons are in bold letters.

**Table 1D. GPCR1b Nucleic acid sequence (SEQ ID NO:3).**

```

TCGCCATGTACAAACGGGTCGTGCTGCCGCATCGAGGGGGACACCATCTCCAGGTGATGCCGCGCTGCTCATTGTGG
CCTTTGTGCTGGGCGCACTAGGCAATGGGGTCGCCCTGTGTGGTTTCTGCTCCACATGAAGACCTGGAAGCCCAGCA
CTGTTTACCTTTTCAATTGGCCGTGGCTGATTTCTCCTTATGATCTGCCTGCCTTTTCGGACAGACTATTACCTCA
GACGTAGACACTGGGCTTTTGGGGACATTCCCTGCCGAGTGGGGCTCTTACGTTGGCCATGAACAGGGCCGGGAGCA
TCGTGTTCTTACGGTGGTGGCTGCCGACAGGTATTTCAAAGTGGTCCACCCCCACCACGCGGTGAACACTATCTCCA
CCCGGGTGGCGGCTGGCATCGTCTGCACCCCTGTGGGCCCTGGTCATCCTGGGAACAGTGTATCTTTTGTGGAGAACC
ATCTCTGCGTGCAAGAGACGGCCGTCTCCTGTGAGAGCTTCATCATGGAGTCGGCCAATGGCTGGCATGACATCATGT
TCCAGCTGGAGTTCTTTATGCCCTCGGCATCATCTTATTTTGTCTCCTTCAAGATTGTTTGGAGCCTGAGGCGGAGGC
AGCAGCTGGCCAGACAGGCTCGGATGAAGAAGCGACCCGGTTCATCATGGTGGTGGCAATTGTGTTTCATCATGCT
ACCTGCCAGCGTGTCTGCTAGACTCTATTTCTCTGGACGGTGGCCCTCGAGTGCCTGCGATCCCTCTGTCCATGGGG
CCCTGCACATAACCTCAGCTTCACCTACATGAACAGCATGCTGGATCCCTGGTGTATTATTTTCAAGCCCTCCT
TTCCCAAATTTACAAACAGCTCAAAATCTGCAGTCTGAAACCCAAAGCAGCCAGGACACTCAAAAACAAAGGCCGG
AAGAGATGCCAATTTGAAACCTCGGTGCGAGGAGTTCATCAGTGTGGCAAATAGTTTCAAAGCCAGTCTGATGGGC
AATGGGATCCCACATTGTTGAGTGGCACTGAACAA

```

The encoded protein is the same as for GPCR1a and is disclosed above in Table 1B.

#### GPCR1c

A disclosed GPCR1c (also referred to as AC011711\_da1) nucleic acid of 1104 nucleotides found by exon linking is shown in Table 1E. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 60-62 and ending with a TGA codon at nucleotides 1098-1100. Putative untranslated regions 5' to the start codon and 3' to the stop codon are underlined in Table 1E and the start and stop codons are in bold letters.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 530 of 770 bases (68%) identical to a gb:GENBANK-ID:HUMHM74|acc:D10923.1 mRNA from *Homo sapiens* (Human mRNA for HM74).

**Table 1E. GPCR1c Nucleic acid sequence (SEQ ID NO:4).**

```

GTGCCATTGTGGGGAAGTCCCTGGGCTGCTCTGCACCGGACACTTGCTCTGTCCCGCCATGTACAACGGGTGCTGCTG
CCGCATCGAGGGGACACCATCTCCAGGTGATGCCGCCGCTGCTCATTGTGGCCTTTGTGCTGGGCGCACTAGACAAT
GGGGTCGCCCTGTGTGGTTTCTGCTTCCACATGAAGACCTGGAAGCCCAGCACTGTTTACCTTTTCAATTTGGCCGTGG
CTGATTTCTCTCTATGATCTGCCTGCTTTTCGGACAGACTATTACCTCAGACGTAGACACTGGGCTTTTGGGGACAT
TCCCTGCCGAGTGGGGCTCTTACGTTGGCCATGAACAGGGCCGGGAGCATCGTGTTCCTTACGGTGGTGGCTGCGGGC
AGGTATTTCAAAGTGGTCCACCCCAACACGCGGTGAACATATCTCCACCGGGTGGCGGTGGCATCGTCTGCACCC
TGTGGGCCCTGGTCATCCTGGGAACAGTGTATCTTTGCTGGAGAACCATCTCTGCGTGCAAGAGACGGCCGTCTCCTG
TGAGAGCTTCATCATGGAGTCGGCCAATGGCTGGCATGACATCATGTTCAGCTGGAGTTCTTTATGCCCTCGGCATC
ATCTTATTTTGTCTCTTCAAGATTGTTTGGAGCCTGAGGCGGAGGACAGCTGGCCAGACAGGCTCGGATGAAGAAGG
CGACCGGGTTCATCATGGTGGTGGCAATTGTGTTTCATCATGCTACCTGCCAGCGTGTCTGCTAGACTCTATTTCTCT
CTGGACGGTCCCTCGAGTGCCTGCGATCCCTCTGTCCATGGGGCCCTGCACATAACCCTCAGCTTCACCTACATGAAC
AGCATGCTGGATCCCTGGTGTATTATTTTCAAGCCCTCTTTCCCAAATTCTACAACAAGCTCAAATCTGCAGTC
TGAACCCCAAGCAGCCAGGACACTCAAAACACAAAGGCCGAAGAGATGCCAATTTCGAACCTCGGTGCGCAGGAGTTG
CATCAGTGTGGCAATAGTTTCAAAGCCAGTCTGATGGGAATGGGATCCCAATTGTTGAGTGGCACTGAACAA

```

The disclosed GPCR1c protein having 346 amino acid residues is presented using the one-letter code in Table 1F. An analysis using the PSORT program predicts that the AC011711\_da1 protein localizes in the plasma membrane with a certainty=0.6400. It is also predicted that protein has a signal peptide whose most likely cleavage site is between residues 36 and 37: GVA-LC in Table 1F.

**Table 1F. Encoded GPCR1c protein sequence (SEQ ID NO:5).**

```

MYNGSCCRIEGDTISQVMPPLLI VAFVLGALDNGVALCGFCFHMKTWKPSTVYLFNLAVADFLLMICLPFR
TDYYLRRRHWAFGDI PCRVGLFTLAMNRAGSIVFLTVVAGRYFKVVHPHNAVNTISTRVAGIVCTLWAL
VILGTVYLLLENHL CVQETAVSCSEFIMESANGWHDIMFQLEFFMPLGI ILFCSEFKIVWSLRRRQQLARQA
RMKKATRFIMVVAIVFITCYLPVSARLYFLWTVPSACDPSVHGALHITLSFTYMNSMLDPLVYFFSSPS
FPKFYNKLKICSLKPKQPGHSKTQRP EEMPISNLGRRSCISVANFSQSDGQWDPHIVEWH

```

A BLASTX search was performed against public protein databases. The full amino acid sequence of the protein of the invention was found to have 270 of 317 amino acid residues 176 of 339 amino acid residues (51%) identical to, and 225 of 339 amino acid residues (66%) similar to, the 387 amino acid residue ptmr:SWISSPROT-ACC:P49019 protein from *Homo sapiens* (Human) (PROBABLE G PROTEIN-COUPLED RECEPTOR HM74). In the following positions, one or more consensus positions of the nucleotide sequence have been identified as single nucleotide polymorphisms (SNPs). "Depth" represents the number of clones covering the region of the SNP. The Putative Allele Frequency (PAF) is the fraction of all the clones containing the SNP. The sign ">" means "is changed to". Possible SNPs found for GPCR1c are listed in Table 1G.

Table 1G: SNPs			
Consensus Position	Depth	Base Change	PAF
334	71	T>C	0.028
677	92	T>C	0.022
719	79	T>C	0.025

The amino acids differences between the three GPCR1 proteins are shown in Table 1H. Deletions are marked by a delta ( $\Delta$ ). The differences between the three proteins appear to be localized to a few distinct regions. Thus, these proteins may have similar functions, such as serving as olfactory or chemokine receptors (see below).

Table 1H. Differences for GPCR1 Proteins		
Position	32	112
GPCR1a	G	D
GPCR1c	D	G

A ClustalW analysis comparing disclosed proteins of the invention with related OR protein sequences is given in Table 1I, with GPCR1a shown on line 1, and GPCR1c on line 2.

In the ClustalW alignment of the GPCR1a protein, as well as all other ClustalW analyses herein, the black outlined amino acid residues indicate regions of conserved sequence (i.e., regions that may be required to preserve structural or functional properties), whereas non-highlighted amino acid residues are less conserved and can potentially be mutated to a much broader extent without altering protein structure or function. Unless specifically addressed as GPCR1a GPCR1b, or GPCR1c, any reference to GPCR1 is assumed to encompass all variants. Residue differences between any GPCRX variant sequences herein are written to show the residue in the "a" variant and the residue position with respect to the "a" variant. GPCR residues in all following sequence alignments that differ between the individual GPCR variants are highlighted with a box and marked with the (o) symbol above the variant residue in all alignments herein. All GPCR1 proteins have significant homology to olfactory receptor (OR) proteins:.

Table 11. ClustalW Analysis of GPCR1

- 1) Novel GPCR1a (SEQ ID NO:2)  
 2) Novel GPCR1c (SEQ ID NO: 5)  
 3) gi|2135386|pir|I69202 G protein-coupled receptor HM74 - human (SEQ ID NO:43)  
 4) gi|11558404|emb|CAC17790.1 (AJ300198) putative seven transmembrane spanning receptor [Mus musculus] (SEQ ID NO:44)  
 5) gi|6716509|gb|AAAF26668.1|AF140708 (AF140708) G protein coupled receptor [Mus musculus] (SEQ ID NO:45)  
 6) gi|464327|sp|P34996|P2YR\_CHICK P2Y PURINOCEPTOR 1 (ATP RECEPTOR) (P2Y1) (PURINERGIC RECEPTOR) (SEQ ID NO:46)  
 7) gi|497261|gb|AAA18784.1 (U09842) P2Y purinergic receptor [Meleagris gallopavo] (SEQ ID NO:47)

		10	20	30	40	50	60
5	GPCR1a	.....	.....	.....	.....	.....	.....
	GPCR1c	.....	.....	.....	.....	.....	.....
	gi 2135386	.....	.....	.....	.....	.....	.....
	gi 11558404	.....	.....	.....	.....	.....	.....
	gi 6716509	.....	.....	.....	.....	.....	.....
10	gi 464327	.....	.....	.....	.....	.....	.....
	gi 497261	.....	.....	.....	.....	.....	.....
		70	80	90	100	110	120
15	GPCR1a	.....	.....	.....	.....	.....	.....
	GPCR1c	.....	.....	.....	.....	.....	.....
	gi 2135386	.....	.....	.....	.....	.....	.....
	gi 11558404	.....	.....	.....	.....	.....	.....
	gi 6716509	.....	.....	.....	.....	.....	.....
10	gi 464327	.....	.....	.....	.....	.....	.....
	gi 497261	.....	.....	.....	.....	.....	.....
		130	140	150	160	170	180
15	GPCR1a	.....	.....	.....	.....	.....	.....
	GPCR1c	.....	.....	.....	.....	.....	.....
	gi 2135386	.....	.....	.....	.....	.....	.....
	gi 11558404	.....	.....	.....	.....	.....	.....
	gi 6716509	.....	.....	.....	.....	.....	.....
10	gi 464327	.....	.....	.....	.....	.....	.....
	gi 497261	.....	.....	.....	.....	.....	.....
		190	200	210	220	230	240
15	GPCR1a	.....	.....	.....	.....	.....	.....
	GPCR1c	.....	.....	.....	.....	.....	.....
	gi 2135386	.....	.....	.....	.....	.....	.....
	gi 11558404	.....	.....	.....	.....	.....	.....
	gi 6716509	.....	.....	.....	.....	.....	.....
10	gi 464327	.....	.....	.....	.....	.....	.....
	gi 497261	.....	.....	.....	.....	.....	.....
		250	260	270	280	290	300
15	GPCR1a	.....	.....	.....	.....	.....	.....
	GPCR1c	.....	.....	.....	.....	.....	.....
	gi 2135386	.....	.....	.....	.....	.....	.....
	gi 11558404	.....	.....	.....	.....	.....	.....
	gi 6716509	.....	.....	.....	.....	.....	.....
10	gi 464327	.....	.....	.....	.....	.....	.....
	gi 497261	.....	.....	.....	.....	.....	.....
		310	320	330	340	350	360
15	GPCR1a	.....	.....	.....	.....	.....	.....
	GPCR1c	.....	.....	.....	.....	.....	.....
	gi 2135386	.....	.....	.....	.....	.....	.....
	gi 11558404	.....	.....	.....	.....	.....	.....
	gi 6716509	.....	.....	.....	.....	.....	.....
10	gi 464327	.....	.....	.....	.....	.....	.....
	gi 497261	.....	.....	.....	.....	.....	.....
		370	380	390	400		

5

10

25

gnl|Pfam|pfam00001, 7tm\_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID NO:71) Length: 254  
Score = 114 bits (284), Expect = 1e-26

30

35

40

45

The 7 transmembrane receptor family includes a number of different proteins, including, for example, serotonin receptors, dopamine receptors, histamine receptors, andrenergic receptors, cannabinoid receptors, angiotensin II receptors, chemokine receptors, opioid receptors, G-protein coupled receptor (GPCR) proteins, olfactory receptors (OR), and the like. Some proteins and the Protein Data Base Ids/gene indexes include, for example: rhodopsin (129209); 5-hydroxytryptamine receptors; (112821, 8488960, 112805, 231454, 1168221, 398971, 112806); G protein-coupled receptors (119130, 543823, 1730143, 132206, 137159, 6136153, 416926, 1169881, 136882, 134079); gustatory receptors (544463, 462208); c-x-c chemokine receptors (416718, 128999, 416802, 548703, 1352335); opsins (129193, 129197, 129203); and olfactory receptor-like proteins (129091, 1171893, 400672, 548417);

GPCR1 is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, MHC II abnd III expressing cells, nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to proprietary database sources, Public EST sources, Literature sources, and/or RACE sources.

The nucleic acids and proteins of GPCR1 are useful in potential therapeutic applications implicated in various GPCR- or olfactory receptor (OR)-related pathologies and/or disorders. For example, a cDNA encoding the G-protein coupled receptor-like protein may be useful in gene therapy, and the G-protein coupled receptor-like protein may be useful when administered to a subject in need thereof. The novel nucleic acid encoding GPCR1 protein, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. The GPCR1 nucleic acids and proteins are useful in potential diagnostic and therapeutic applications implicated in

various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: developmental diseases, MHCII and III diseases (immune diseases), Taste and scent detectability Disorders, Burkitt's lymphoma, Corticoneurogenic disease, Signal Transduction pathway disorders, Retinal diseases including those involving photoreception, Cell Growth rate disorders; Cell Shape disorders, Feeding disorders; control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidoluysian atrophy(DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from neoplasm, adenocarcinoma, lymphoma, prostate cancer, uterus cancer, immune response, AIDS, asthma, Crohn's disease, multiple sclerosis, and Albright Hereditary Osteodystrophy. Additional GPCR-related diseases and disorders are mentioned throughout the Specification.

Further, the protein similarity information, expression pattern, and map location for GPCR1 suggests that GPCR1 may have important structural and/or physiological functions characteristic of the GPCR family. The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding GPCR1 may be useful in gene therapy, and GPCR1 may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary

retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and  
5 dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding OR-like protein, and the OR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the  
10 novel substances of the invention for use in therapeutic or diagnostic methods.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR1 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR1 Antibodies" section below.

15 The disclosed GPCR1 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR1 epitope is from about amino acids 1 to 10. In another embodiment, a GPCR1 epitope is from about amino acids 75 to 100. In additional embodiments, GPCR1 epitopes are from amino acids 130 to 140, 210-230, and from amino acids 270 to 330. These novel proteins can also be used to develop assay system  
20 for functional analysis.

## GPCR2

An additional GPCR-like protein of the invention, referred to herein as GPCR2, is an Olfactory Receptor ("OR")-like protein. The novel nucleic acid of 1149 nucleotides, mapped to chromosome 5, (80250319\_EXT, SEQ ID NO:6) encoding a novel G-protein coupled  
25 receptor-like protein is shown in Table 2A.



**Table 2A. GPCR2 Nucleotide Sequence (SEQ ID NO:6)**

```

ATGGCCGATGCAGCCACGATAGCCACCATGAATAAGGCAGCAGGCGGGGACAAGCTAGCAGAACTCTTCAGTCTGGT
CCCGGACCTTCTGGAGGCGGCCAACACGAGTGGTAACGCGTCGCTGCAGCTCCGGACTTGTGGTGGGAGCTGGGGC
TGGAGTTGCCGAGCGCGCGCCGCCAGACATCCCCGGGCGAGCGGGGGCAGAGAGCGCGGACACAGAGGCCCGG
GTGCGGATTCTCATCAGCGTGGTGTACTGGGTGGTGTGCGCCCTGGGGTTGGCGGGCAACCTGCTGGTTCTTACCT
GATGAAGAGCATGCAGGGCTGGCGCAAGTCCCTCTATCAACCTCTTCGTCAACCACTGGCGCTGACGGACTTTCAGT
TTGTGCTCACCTGCCCTTCTGGGCGGTGGAGAAGCTCTTGACTTCAAATGGCCCTTCGGCAAGGCCATGTGAAG
ATCGTGTCCATGGTGACGTCCATGAACATGTACGCCAGCGTGTCTTCCCTCACTGCCATGAGTGTGACGCGCTACCA
TTCGTGGCCTCGGCTCTGAAGAGCCACCGGACCCGAGGACACGGCCGGGGCGACTGCTGGGCCGGAGCCTGGGGG
ACAGCTGCTGCTTCTCGGCCAAGGCGCTGTGTGTGGATCTGGGCTTTGGCCGCGCTGGCCTCGCTGCCAGTGCC
ATTTTCTCCACCACGGTCAAGGTGATGGGCGAGGAGCTGTGCACTGGTGCCTTCCCGACAAGTTGCTGGGCCGCG
ACAGGCAGTTCTGGCTGGGCTCTACCACTCGCAGAAGAAGCTGCTGGGGTACCGGCTTACTTAGCATATATTTTAA
TTCCAAAACAATTCTTTAGATCACTACCTCTTTCTTACGACCTCTTGATTTTCCGCCCTCTCTTACCCTTCCGTT
ATCCGCAACATTTCTTCTTACCGCCACAACGATAAACCGCGTAGGACCTGGTGTCCACCCCATGGACTGGACC
CGCCAGTCCAGACCAGATTGAAATACGTATAGATTGCTACCTGCTATGTACATCACTATGAATTTCTGGCATTTA
AATCAAACAGATTTTCAGGAAGTAGCCTGGGGACTCAGACACCATTAAACCTTGGGAAAGCATGTTTTGA

```

An open reading frame (ORF) for GPCR2 was identified from nucleotides 1 to 1146. The disclosed GPCR2 polypeptide (SEQ ID NO:7) encoded by SEQ ID NO:6 is 382 amino acid residues with a molecular weight of 41669.7 Daltons and is presented using the one-letter code in Table 2B. The GPCR2 protein was analyzed for signal peptide prediction and cellular localization. Psort and Hydropathy profiles predict that GPCR2 contains a signal peptide and is likely to be localized at the plasma membrane (certainty of 0.6000).

**Table 2B. Encoded GPCR2 protein sequence (SEQ ID NO:7).**

```

MADAATIATMNKAAGGDKLAELFSLVPLDLEAANTSGNASLQLPDLWWEIGLELPDGAAPPGHPPGSGGAESADTEAR
VRILISVVYVWVVCALGLAGNLLVLYLMKSMQGWKSSINLFVTNLALTDQFVLTLFPWAVENALDFKWPFGKAMCK
IVSMVTSMNMYASVFFLTAMSVTRYHSVASALKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIWALAALASLPASA
IFSTTVKVMGEELCTGAFFGQVAGPRQAVLAGPLPLAEAAAGVPAYLAYIFIPKQFFRSLPLSYDLLYFPPLSYPSV
IRNISSLPPQHDKPRRTWCFFPWTPASPDQIENTYRFATCYVHHYEFALFKSNRFSGLTSLGTQTPFKPWESMF

```

The GPCR2 nucleic acid sequence has 405 of 648 nucleotides (62%) identical to Sequence 9 from patent US 5436155 (GENBANK-ID:I13406)

The full amino acid sequence of the protein of the invention was found to have 62 of 170 amino acid residues (36%) identical to, and 96 of 170 amino acid residues (56%) positive with, the 359 amino acid residue protein from *Rattus norvegicus* (SWISSPROT-ACC:P29089). The protein encoded by GPCR2 (SEQ ID NO:6) has significant homology to olfactory, odorant, and taste chemoreceptors and belongs to the family of G-Protein coupled receptors (GPCRs). This family of genes has been used as a target for small molecule drugs and GPCRs are expressed on the plasma membrane and are also a suitable target for protein drugs like therapeutic antibodies, cytotoxic antibodies and diagnostic antibodies.

Another BLAST against GenBank Accession Number: XP\_003874.1, a 471 amino acid G-protein coupled receptor SALPR; somatostatin and angiotensin-like peptide receptor protein from *Homo sapiens*, produced 100% identity, between a 245 fragment and amino acids 1-247 of GPCR2 (Table 2C).

5

Table 2C: Alignment of GPCR2 with gi 11416964 ref XP_003874.1  G-protein coupled receptor SALPR; somatostatin and angiotensin-like peptide receptor [Homo sapiens] (SEQ ID NO:48)	
Score = 396 bits (1018), Expect = e-109 Identities = 207/255 (81%), Positives = 209/255 (81%)	
	10 20 30 40 50 60
GPCR2	..MADAATIATMNKAAGGDKLAELFSLVDPDLLEAANTSGNASLQLPDLWELGLELPDGA
gi 11416964	..MADAATIATMNKAAGGDKLAELFSLVDPDLLEAANTSGNASLQLPDLWELGLELPDGA
	70 80 90 100 110 120
GPCR2	PPGHPPGSGGAESADTEARVRILISVVYVWVVCALGLAGNLLVLYLNKSMQGWKSSINLF
gi 11416964	PPGHPPGSGGAESADTEARVRILISVVYVWVVCALGLAGNLLVLYLNKSMQGWKSSINLF
	130 140 150 160 170 180
GPCR2	VTNLALTDQFVLTLPFWAVENALDFKWPFGKAMCKIVSMVTSMNNYASVFFLTAMSVTR
gi 11416964	VTNLALTDQFVLTLPFWAVENALDFKWPFGKAMCKIVSMVTSMNNYASVFFLTAMSVTR
	190 200 210 220 230 240
GPCR2	YHSVASALKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIIWALAALASLPSAIFSTTVK
gi 11416964	YHSVASALKSHRTRGHGRGDCCGRSLGDSCCFSAKALCVWIIWALAALASLPSAIFSTTVK
	250 260 270 280 290 300
GPCR2	VMGEELCTGAFEGGVAC-----PRQAVTAGFLPL-----
gi 11416964	VMGEELCLVRFFDKHLGRDQFVWGLYHSSKVLICFVLPGLGIILCYLLVRFIADRRAA
	310 320 330 340 350 360
GPCR2	---EEESAG---VPAYLAYHFIKPFKFFRSQPLSDLLAFI-----SLK
gi 11416964	GTKGGKAVNGGRPTGASARRSKVTNSVTINVLSEFFCILENQALTTSILIKFNAVEFS
	370 380 390 400 410 420
GPCR2	YPSVERNTSSLEFQHDKPRRTWPPPTWTGPASPDIENTYHEATCYVHH--YEFLKPK
gi 11416964	QEYFECQYRFFVSVCLAHSNSLNEVLYCLVRREFRKALSSLLWRDAS--PSITSMRPF
	430 440 450 460 470
GPCR2	---ENRFSGTSLSTWDFKWEEMF-----
gi 11416964	TATTKPEHEDQGLADAPLHAHAEPDLLYPPGVVYSGGRYDLLPSSSAY

Other BLAST results including the sequences used for ClustalW analysis are presented in Table 2D. Other BLAST alignment data is shown in Table 2E.

**Table 2D. BLAST results for GPCR2**

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
Gi 12188901 emb CAC21550.1  (AJ301623)	angiotensin II type 1 receptor [Cavia porcellus]	359	46/109 (42%)	61/109 (55%)	6e-16
>gi 8927995 sp Q9WV26 AG2R_CAVPO	TYPE-1 ANGIOTENSIN II RECEPTOR (AT1) [Cavia porcellus]	359	45/109 (41%)	61/109 (55%)	1e-15
Gi 90297 pir  JH0621	angiotensin II receptor 1A - mouse	359	44/109 (40%)	61/109 (55%)	2e-15
Gi 249946 gb AAB22269.1  (S37484)	Angiotensin II receptor isoform 1a, AT-1a receptor Balb/c, liver	359	44/109 (40%)	61/109 (55%)	2e-15

**Table 2E. BLASTX alignments of GPCR2**

Sequences producing High-scoring Segment Pairs:				Smallest Sum
		Reading Frame	High Score	Prob. P(N)
patp:Y92934	Human G-protein coupled receptor clone hOT	+1	1297	6.1e-145
patp:Y92932	Rat G-protein coupled receptor clone rOT7T	+1	1134	1.7e-125
patp:Y92933	Rat G-protein coupled receptor clone rOT7T	+1	1134	1.7e-125
patp:W92645	Human membrane penetrating type receptor p	+1	314	2.4e-27

- This information is presented graphically in the multiple sequence alignment given in
- 5 Table 2F (with GPCR2 being shown on line 1) as a ClustalW analysis comparing GPCR2 with related protein sequences.

**Table 2F. Information for the ClustalW proteins:**

- 10 1) Novel GPCR2 (SEQ ID NO:6)
- 2) gi|11416964|ref|XP\_003874.1| G-protein coupled receptor SALPR; somatostatin and angiotensin-like peptide receptor [Homo sapiens] (SEQ ID NO:48)
- 3) gi|12188901|emb|CAC21550.1| (AJ301623) angiotensin II type 1 receptor [Cavia porcellus] (SEQ ID NO:49)
- 15 4) gi|8927995|sp|Q9WV26|AG2R\_CAVPO TYPE-1 ANGIOTENSIN II RECEPTOR (AT1) (SEQ ID NO:50)
- 5) gi|90297|pir||JH0621 angiotensin II receptor 1A - mouse (SEQ ID NO:51)
- 6) gi|249946|gb|AAB22269.1| (S37484) angiotensin II receptor isoform 1a, AT-1a receptor (SEQ ID NO:52)

	10	20	30	40	50	60
GPCR2	.....	.....	.....	.....	.....	.....
gi 11416964	--MADAATATMNRKAGGDKLAELFSLVPDLLEAANTSGNASLQLPDLWWELGLELPDGA					
gi 12188901	-----MLNSST-----					QGIKRIQDC

```

gi|8927995|      . . . . . YILNSST      . . . . . EDGIKRIQDD
gi|90297|        . . . . . YALNSST      . . . . . EDGIKRIQDD
gi|249946|      . . . . . YALNSST      . . . . . EDGIKRIQDD

          70          80          90          100          110          120
GPCR2      PEGHPPGSGGAESADTEARRRLLISVWVWVYCAIGAGNLVVIYLMKSMQGRWRSSITLLE
gi|11416964| PEGHPPGSGGAESADTEARRRLLISVWVWVYCAIGAGNLVVIYLMKSMQGRWRSSITLLE
gi|12188901| CPKD--GR      HSYIFVMIPTLYSIIFVVGIFGNSLVVIVIFYFMKL--KTVASVF
gi|8927995|  CPKA--GR      HSYIFVMIPTLYSIIFVVGIFGNSLVVIVIFYFMKL--KTVASVF
gi|90297|    CPKA--GR      HSYIFVMIPTLYSIIFVVGIFGNSLVVIVIFYFMKL--KTVASVF
gi|249946|  CPKA--GR      HSYIFVMIPTLYSIIFVVGIFGNSLVVIVIFYFMKL--KTVASVF

          130          140          150          160          170          180
GPCR2      VTNLALADDFQVLTLPFAVENALDFKWPFGKALCKIVSMVTSSNMVYASVFLTAMSVTF
gi|11416964| VTNLALADDFQVLTLPFAVENALDFKWPFGKALCKIVSMVTSSNMVYASVFLTAMSVTF
gi|12188901| LLNLALADICFLLTLPMAVYTAMEYRWPFGNMLCKIASASVSFNLYASVFLLTCLSIDR
gi|8927995|  LLNLALADICFLLTLPMAVYTAMEYRWPFGNMLCKIASASVSFNLYASVFLLTCLSIDR
gi|90297|    LLNLALADICFLLTLPMAVYTAMEYRWPFGNMLCKIASASVSFNLYASVFLLTCLSIDR
gi|249946|  LLNLALADICFLLTLPMAVYTAMEYRWPFGNMLCKIASASVSFNLYASVFLLTCLSIDR

          190          200          210          220          230          240
GPCR2      YHSVASALKSHRTGCHGRGDCCGRSLGDSCCFSAKALCVMIWAKAAGLASLPSAIFSTTVK
gi|11416964| YHSVASALKSHRTGCHGRGDCCGRSLGDSCCFSAKALCVMIWAKAAGLASLPSAIFSTTVK
gi|12188901| YLAIVHPMKSLRRTV-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF
gi|8927995|  YLAIVHPMKSLRRTV-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF
gi|90297|    YLAIVHPMKSLRRTV-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF
gi|249946|  YLAIVHPMKSLRRTV-----LVAKVTCVIIWLMAGLASLPAVIHRNVVF

          250          260          270          280          290          300
GPCR2      VMGEECTGAPPCFVAG-----PRDAVLAGPLPL-----
gi|11416964| VMGEECTGAPPCFVAG-----PRDAVLAGPLPL-----
gi|12188901| IENTNITVCAFHYESNSTLPIGLG--LTKNILGFMFPFLIILTSY--
gi|8927995|  IENTNITVCAFHYESNSTLPIGLG--LTKNILGFMFPFLIILTSY--
gi|90297|    IENTNITVCAFHYESNSTLPIGLG--LTKNILGFMFPFLIILTSY--
gi|249946|  IENTNITVCAFHYESNSTLPIGLG--LTKNILGFMFPFLIILTSY--

          310          320          330          340          350          360
GPCR2      -----AEEAAG--PAPLAYIIFPQFFRSLSLMDLLMFE-----PLS
gi|11416964| GTKGGAAGVAGRGPTGABANLSKPTFSYIIVLSGLFGLNLTALITWSLLEFNAPFFS
gi|12188901| TLIWKALKKKAYEIQKNKPRNDIDFRIIMAIVLVFFFSWVPHQIETFLDVLVLIQLG--LIHQ
gi|8927995|  TLIWKALKKKAYEIQKNKPRNDIDFRIIMAIVLVFFFSWVPHQIETFLDVLVLIQLG--LIHQ
gi|90297|    TLIWKALKKKAYEIQKNKPRNDIDFRIIMAIVLVFFFSWVPHQIETFLDVLVLIQLG--LIHQ
gi|249946|  TLIWKALKKKAYEIQKNKPRNDIDFRIIMAIVLVFFFSWVPHQIETFLDVLVLIQLG--LIHQ

          370          380          390          400          410          420
GPCR2      YPSVIRINISLPPQHDKPRRTWPPPWTPGASPDIDNTKRFATCVVHH--YEFLAFK-
gi|11416964| QHYELCOVYAPPEVYCLAHSNCLNPLFLCLRRERFALKSLNRRAS--PSLTSMRPF
gi|12188901| CKISDIVDTAMPPTICIAVENNCLNPLFYGFLGKKFKKYYFLQLLKYIPPAKSHSLSTK
gi|8927995|  CKISDIVDTAMPPTICIAVENNCLNPLFYGFLGKKFKKYYFLQLLKYIPPAKSHSLSTK
gi|90297|    CKISDIVDTAMPPTICIAVENNCLNPLFYGFLGKKFKKYYFLQLLKYIPPAKSHSLSTK
gi|249946|  CKISDIVDTAMPPTICIAVENNCLNPLFYGFLGKKFKKYYFLQLLKYIPPAKSHSLSTK

          430          440          450          460          470
GPCR2      -----SRRSCSLGCTPPKWEESMF-----
gi|11416964| TATTKPEHEDGGLQAPAPPAHAAEDLLLYPPGVVVYSGGRYDLLPSSSAY
gi|12188901| MSTLSYRPSDNVSSSAKKPVQCEVE-----
gi|8927995|  MSTLSYRPSDNVSSSAKKPVQCEVE-----
gi|90297|    MSTLSYRPSDNVSSSAKKPVQCEVE-----
gi|249946|  MSTLSYRPSDNVSSSAKKPVQCEVE-----

```

DOMAIN results for GPCR2 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The 7tm 1, a seven transmembrane receptor (rhodopsin family),

was shown to have homology to GPCR2. ( $E=8e-20$ ). This indicates that the GPCR2 sequence has properties similar to those of other proteins known to contain this domain as well as to the 7tm\_1 domain itself (Table 2G).

**Table 2G. Domain Analysis of GPCR2**

gnl|Pfam|pfam00001, 7tm\_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID NO:72) Length: 254  
CD-Length = 254 residues, only 50.0% aligned  
Score = 91.3 bits (225), Expect = 8e-20

```

5      10      20      30      40      50      60
GPCR2 (SEQ ID NO:7) -NLLVHYIKMSMGWPK-ESINILEYINLAIIDFQVLTLPFAVENAIDFKNFEGKAYCK
Consensus 7tm 1 domain GNLLVLLILRLTRKLP-EPINIDILNLAYACILFLFETLPFWAHYYILGGDIVEGDAECK

10      70      80      90      100     110     120
GPCR2 -VSMVTSINMYASFFLTANSEITRVHGLASAKKSHITFGHGFGDCCGRSLGDSCCFSAKA
Consensus 7tm 1 domain -LVGALEFVNGYASILLLTANSLIDRYLIVHPELYRPIPTPRP-----ASV

15      130     140     150     160     170     180
GPCR2 -LCWVTHALLAALASLSAIESITTVKVMGEE-----ETGAEEGVVAGP-FOAVLAGPFP
Consensus 7tm 1 domain -LTLVLEVLLALLSLEPLIFSRLRTVEEGNT-TVALIDSEPSVK--PSYVLLST-

20      190     200     210     220     230     240
GPCR2 -LAEAAAGPAYIAYEIFPKPFESPLSYDLLYFPPLSYPSVIRINISSLPPQHDKPRRT
Consensus 7tm 1 domain -----VGFEPLPLIVIVCYTRILPITRKRSQRSRLKRSSSERAAKMLVVVVVFLCP

25      250     260     270     280     290
GPCR2 -CPPPWGTGASPDQDEITM-----FATCYHHYELAEKSNRFETSLGTQTPFKP
Consensus 7tm 1 domain -LEYHIVLLLDLSCLSLIP-----VLPTAELITLNLIVGCLPIIY-----

```

Based on information available on expression of SWISSPROT-ACC:P29089 TYPE-1B ANGIOTENSIN II RECEPTOR (AT1B) (AT3) - *Rattus norvegicus* (Rat), the closest G-Protein Coupled Receptor family member it is likely that GPCR2 is expressed in cardiac tissue, renal tissue, and vascular tissue as angiotensin is expressed in these tissues.

GPCR2 includes the nucleic acid whose sequence is provided in Table 2A, or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 2A while still encoding a protein that maintains its G-Protein Coupled Receptor-like activities and physiological functions, or a fragment of such a nucleic acid. GPCR2 further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. GPCR2 additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in

therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 38 percent of the bases may be so changed.

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor -like protein may be useful in gene therapy, and the olfactory receptor -like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from Cardiovascular disorders, Hypertension, Diabetes, Autoimmune disease, Renal artery stenosis, Interstitial nephritis, Glomerulonephritis, Polycystic kidney disease, Systemic lupus erythematosus, Renal tubular acidosis, IgA nephropathy, Hypercalcaemia, Lesch-Nyhan syndrome, Cardiomyopathy, Atherosclerosis, Hypertension, Congenital heart defects, Aortic stenosis, Atrial septal defect (ASD), Atrioventricular (A-V) canal defect, Ductus arteriosus, Pulmonary stenosis, Subaortic stenosis, Ventricular septal defect (VSD), valve diseases, Tuberosus sclerosis, Scleroderma, Obesity, Cell signalling disorders, Cancer, Muscular degeneration, Metabolic and Endocrine disorders, Respiratory disorders, Tissue/Cell growth regulation disorders, and Developmental disorders. Other GPCR-related diseases and disorders are contemplated.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. The disclosed GPCR2 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR2 epitope is from about amino acids 50 to 90. In another embodiment, a GPCR2 epitope is from about amino acids 180 to 230. In an additional embodiment, GPCR2 epitopes are from amino acids 310 to 360.

These novel proteins can be used in assay systems for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

**GPCR3**

An additional GPCR-like protein of the invention, referred to herein as GPCR3, is an Olfactory Receptor ("OR")-like protein. The novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probe or homolog, run  
 5 against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The novel nucleic acid of 970 nucleotides (AC020597, SEQ ID NO:8)  
 10 encoding a novel olfactory receptor-like protein is shown in Table 3A. An open reading frame (ORF) was identified beginning with an ATG initiation codon at nucleotides 27-29 and ending with a TAA codon at nucleotides 947-949. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 3A, and the start and stop codons are in bold letters.

**Table 3A. GPCR3 Nucleotide Sequence (SEQ ID NO:8)**

```

AAAAAGTTCCCAGAAGAACGGCCTCAATGAATACCACTCTATTTATCCTTACTCTTTCCTTCTTCTGGGAATTC
CTGGGCTGGAAAGTATGCATCTCTGGGTTGGTTTTCTTTCTTTGCTGTGTTCTTGACAGCTGTCCTTGGGAATA
TCACCATCCTTTTGTGATTTCAGACTGACAGTAGTCTCCATCATCCCATGTTCTACTTCTTGCCCATTCGTGCAT
CTATTGACCCGGGCTGTCTACATCCACCATCCCTAAATGCTTGGCACCTTCTGGTTTACCCTGAGAGAAATCT
CCTTTGAAGGATGCCTTACCCAGATGTTCTTCATCCACCTGTGCACTGGCATGGAATCAGCTGTGCTTGTGGCCA
TGGCCTATGATTGCTATGTGGCCATCTGTGACCCCTCTTTGCTACACGTTGGTGCTGACAAACAAGGTGGTGTCAG
TTATGGCACTGGCCATCTTTCTGAGACCCCTTAGTCTTTGTCATACCCCTTGTCTATTATCCTAAGGCTTCCAT
TTTGTGGACACCAATATTCTCTACTTATGGTGAGCACATGGGCATTGCCCGCTGTCTGTGCCAGCATCA
GGGTAAACATCATCTATGGCTTATGTGCATCTCTATCCTGGTCTTTGACATCATAGCAATTGTCATTTCTATG
TACAGATCCTTTGTGCTGTATTTCTACTCTCTTCACATGATGCACGACTCAAGGCATTGACACCTGTGGCTCTC
ATGTGTGTGCATGTTGACTTTCTATATGCGTGCATTTTCTCATTTCATGACCCATAGGTTTGGTCGGAATATAC
CTCACTTTATCCACATTCTTCTGGCTAATTTCTATGTAGTCAATCCACCTGCTCTCAACTCTGTAATTTATGGTG
15 TCAGAACCAACAGATTAGAGCACAGTGCTGAAATGTTTTCAATAAATAAACATAGCTCATTATA

```

In a search of sequence databases, it was found, for example, that the nucleic acid sequence of GPCR3 has 627 of 904 bases (69%) identical to a *Mus musculus* odorant receptor S46 gene (GENBANK-ID: AF121979).

The disclosed GPCR3 polypeptide (SEQ ID NO:9) encoded by SEQ ID NO:8 is 308  
 20 amino acid residues, a molecular weight of 34713.3 Daltons and is presented using the one-letter code in Table 3B. The GPCR3 protein were analyzed for signal peptide prediction and cellular localization. SignalP results predict that GPCR3 is cleaved between position 40 and 41 of SEQ ID NO:10, *i.e.*, at the slash in the amino acid sequence VLG-NI. Psort and Hydropathy profiles also predict that GPCR3 contains a signal peptide and is likely to be  
 25 localized at the plasma membrane (certainty of 0.6400).

**Table 3B. Encoded GPCR3 protein sequence (SEQ ID NO:9).**

MNTTLFHPYSFLLGIPGLESMLWVGFPFFAVFLTAVLGNITILFVIQTDSSLHHPMFYFLAILSSIDPGLSTS  
 TIPKMLGTFWFTLREISFEGCLTQMFFIHLCTGMESAVLVAMAYDCYVAICDPLCYTLVLTNKVSVMLAIFLR  
 PLVFVIPFVLFILRLPFCGHQIIPHTYGEHMGIALSCASIRVNIYGLCAISILVFDIIAIVISYVQILCAVFL  
 LSSHDARLKAFSTCGSHVCVMLTFYMPAFFSEFMTHRFRNIPHFHILLANFYVVI PPALNSVIYGVRTKQIRAQ  
 VLKMEFFNK

The full amino acid sequence of the protein of the invention was found to have 178 of  
 307 amino acid residues (57%) identical to, and 231 of 307 residues (75%) positive with, the  
 318 amino acid residue protein ODORANT RECEPTOR S46 from - *Mus musculus* (Mouse)  
 5 (ptr:SP TREMBL-ACC: Q9WU93), and 146 of 306 amino acid residues (47%) identical to,  
 and 208 of 306 residues (67%) positive with, the 312 amino acid residue protein Olfactory  
 Receptor HPFH1OR from *Homo sapiens* (TREMBLNEW-ACC:AAD51279).

The disclosed GPCR3 protein (SEQ ID NO:9) also has good identity with a number of  
 olfactory receptor proteins, as shown in Table 3C and 3D.

10

**Table 3C. BLAST results for GPCR3**

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 9935442 ref NP_064688.1	odorant receptor S46 gene [Mus musculus]	318	178/307 (57%)	231/307 (74%)	7e-86
gi 6532001 gb AAD27596.2 AF121976_1 (AF121976)	odorant receptor S19 [Mus musculus]	339	159/291 (54%)	211/291 (71%)	1e-75
Gi 11908211 gb AA41676.1  (AF137396)	HOR 5'Beta14 [Homo sapiens]	318	157/299 (52%)	214/299 (71%)	5e-73
Gi 9938014 ref NP_064686.1	odorant receptor S18 gene [Mus musculus]	321	155/304 (50%)	213/304 (69%)	5e-73
Gi 7305349 ref NP_038647.1	olfactory receptor 67 [Mus musculus]	326	158/297 (53%)	213/297 (71%)	6e-71

**Table 3D. BLASTX alignments of GPCR3**

Sequences producing High-scoring Segment Pairs:				Smallest Sum
	Reading Frame	High Score		Prob. P(N)
Patp:W01730 Human G-protein receptor HPRAJ70 - Homo sa	+3	721	1.8e-70	
Patp:W56641 G-protein coupled prostate tissue receptor	+3	721	1.8e-70	
Patp:Y92365 G protein-coupled receptor protein 5 - Hom	+3	718	3.8e-70	
Patp:R27875 Odorant receptor clone I14 - Rattus rattus	+3	486	1.5e-45	



This information is presented graphically in the multiple sequence alignment given in Table 3E (with GPCR3 being shown on line 1) as a ClustalW analysis comparing GPCR3 with related protein sequences.

**Table 3E. Information for the ClustalW proteins:**

- 1) Novel GPCR3 (SEQ ID NO:9)
- 2) gi|9935442|ref|NP\_064688.1| odorant receptor S46 gene [Mus musculus] (SEQ ID NO:53)
- 3) gi|6532001|gb|AAD27596.2|AF121976\_1 (AF121976) odorant receptor S19 [Mus musculus] (SEQ ID NO:54)
- 4) gi|11908211|gb|AAG41676.1| (AF137396) HOR.5Beta14 [Homo sapiens] (SEQ ID NO:55)
- 5) gi|9938014|ref|NP\_064686.1| odorant receptor S18 gene [Mus musculus] (SEQ ID NO:56)
- 6) gi|7305349|ref|NP\_038647.1| olfactory receptor 67 [Mus musculus] (SEQ ID NO:57)

	10	20	30	40	50	60
GPCR3	..... ..... ..... ..... ..... ..... .....	MNTLLEHYSSTLLGIPGLESMHLEWGPPEP				
gi 9935442	----- ----- ----- ----- ----- ----- -----	MSPGNSSEWIHPSSSLLGIPGLEELQFHLGIPFG				
gi 6532001	----- ----- ----- ----- ----- ----- -----	MPEKMLSKLIAYLLIESCRQAQLVKGRRIVVDSRPHWPNTTHYRELEDOHVLAIIPFC				
gi 11908211	----- ----- ----- ----- ----- ----- -----	MSDSNLSDNHLPTTSELGIPGLEAAHFWLAIIPFC				
gi 9938014	----- ----- ----- ----- ----- ----- -----	MNSKASMLGTNEIITHTVSHLLGIPGLEQYHTLSIPFC				
gi 7305349	----- ----- ----- ----- ----- ----- -----	MKVASSFHHIDNPQDVWVYVLLGIPGLEDLHSWLAIPFC				
	70	80	90	100	110	120
GPCR3	..... ..... ..... ..... ..... ..... .....	RYELTAVVGNVITLTVIOTSSLEHHPNTVEIATLSICPGLSTSTIPKMLCTFWETLREI				
gi 9935442	..... ..... ..... ..... ..... ..... .....	TVYLAVALGNVITLTVIOTSSLEHHPNTVEIATLSICPGLSTSTIPKMLCTFWETLREI				
gi 6532001	..... ..... ..... ..... ..... ..... .....	SMYLAVALGNVITLTVIOTSSLEHHPNTVEIATLSICPGLSTSTIPKMLCTFWETLREI				
gi 11908211	..... ..... ..... ..... ..... ..... .....	SMYLAVALGNVITLTVIOTSSLEHHPNTVEIATLSICPGLSTSTIPKMLCTFWETLREI				
gi 9938014	..... ..... ..... ..... ..... ..... .....	SMYLAVALGNVITLTVIOTSSLEHHPNTVEIATLSICPGLSTSTIPKMLCTFWETLREI				
gi 7305349	..... ..... ..... ..... ..... ..... .....	SMYLAVALGNVITLTVIOTSSLEHHPNTVEIATLSICPGLSTSTIPKMLCTFWETLREI				
	130	140	150	160	170	180
GPCR3	..... ..... ..... ..... ..... ..... .....	SFGGCTTOMFFIHLCTGMESEVLVAMAYDCYVAICDPLCYTLVLTNKVSVNALMIFLPP				
gi 9935442	..... ..... ..... ..... ..... ..... .....	AFRDCVZOMFFIHLCTGMESEVLVAMAYDCYVAICDPLCYTLVLTNKVSVNALMIFLPP				
gi 6532001	..... ..... ..... ..... ..... ..... .....	SYHCCITOMFFIHLCTGMESEVLVAMAYDCYVAICDPLCYTLVLTNKVSVNALMIFLPP				
gi 11908211	..... ..... ..... ..... ..... ..... .....	SFGGCTTOMFFIHLCTGMESEVLVAMAYDCYVAICDPLCYTLVLTNKVSVNALMIFLPP				
gi 9938014	..... ..... ..... ..... ..... ..... .....	PFDACTTOMFFIHLCTGMESEVLVAMAYDCYVAICDPLCYTLVLTNKVSVNALMIFLPP				
gi 7305349	..... ..... ..... ..... ..... ..... .....	SFGGCTTOMFFIHLCTGMESEVLVAMAYDCYVAICDPLCYTLVLTNKVSVNALMIFLPP				
	190	200	210	220	230	240
GPCR3	..... ..... ..... ..... ..... ..... .....	FVSVIPFVILRLPSCGHIIIPHTYCEHMGIAKLACASHKENYGLTVANEVTCMDVY				
gi 9935442	..... ..... ..... ..... ..... ..... .....	FVSVIPFVILRLPSCGHIIIPHTYCEHMGIAKLACASHKENYGLTVANEVTCMDVY				
gi 6532001	..... ..... ..... ..... ..... ..... .....	FVSVIPFVILRLPSCGHIIIPHTYCEHMGIAKLACASHKENYGLTVANEVTCMDVY				
gi 11908211	..... ..... ..... ..... ..... ..... .....	FVSVIPFVILRLPSCGHIIIPHTYCEHMGIAKLACASHKENYGLTVANEVTCMDVY				
gi 9938014	..... ..... ..... ..... ..... ..... .....	FVSVIPFVILRLPSCGHIIIPHTYCEHMGIAKLACASHKENYGLTVANEVTCMDVY				
gi 7305349	..... ..... ..... ..... ..... ..... .....	FVSVIPFVILRLPSCGHIIIPHTYCEHMGIAKLACASHKENYGLTVANEVTCMDVY				
	250	260	270	280	290	300
GPCR3	..... ..... ..... ..... ..... ..... .....	ATVTSYVOILCAVELLSHDAIKALTCGSHVCVMTSYMPAFFSETHRFG-RNTHF				
gi 9935442	..... ..... ..... ..... ..... ..... .....	LSATSYAKILHAEVRLPSWEARLKALNTCGSHVCVMTSYMPAFFSETHRFG-RNTHF				
gi 6532001	..... ..... ..... ..... ..... ..... .....	LIATSYVILCAVELLSHDAIKALTCGSHVCVMTSYMPAFFSETHRFG-RNTHF				
gi 11908211	..... ..... ..... ..... ..... ..... .....	LIATSYVILCAVELLSHDAIKALTCGSHVCVMTSYMPAFFSETHRFG-RNTHF				
gi 9938014	..... ..... ..... ..... ..... ..... .....	LIATSYVILCAVELLSHDAIKALTCGSHVCVMTSYMPAFFSETHRFG-RNTHF				
gi 7305349	..... ..... ..... ..... ..... ..... .....	LIATSYVILCAVELLSHDAIKALTCGSHVCVMTSYMPAFFSETHRFG-RNTHF				
	310	320	330	340		
GPCR3	..... ..... ..... ..... ..... ..... .....	THILLANLYVVPALNSVIYGVTRKQIPADVLYKFFNK-----				
gi 9935442	..... ..... ..... ..... ..... ..... .....	THILLANLYVVPALNSVIYGVTRKQIPADVLYKFFNK-----				
gi 6532001	..... ..... ..... ..... ..... ..... .....	THILLANLYVVPALNSVIYGVTRKQIPADVLYKFFNK-----				
gi 11908211	..... ..... ..... ..... ..... ..... .....	THILLANLYVVPALNSVIYGVTRKQIPADVLYKFFNK-----				
gi 9938014	..... ..... ..... ..... ..... ..... .....	THILLANLYVVPALNSVIYGVTRKQIPADVLYKFFNK-----				
gi 7305349	..... ..... ..... ..... ..... ..... .....	THILLANLYVVPALNSVIYGVTRKQIPADVLYKFFNK-----				

DOMAIN results for GPCR3 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The 7tm\_1, a seven transmembrane receptor (rhodopsin family), was shown to have significant homology to GPCR3. ( $E=7e-11$ ). This indicates that the GPCR3 sequence has properties similar to those of other proteins known to contain this domain as well as to the 7tm\_1 domain itself.

**Table 3F. Domain Analysis of GPCR3**

gnl|Pfam|pfam00001, 7tm\_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID NO:73) Length: 254  
CD-Length = 254 residues, 100.0% aligned  
Score = 61.2 bits (147), Expect =  $7e-11$

		10	20	30	40	50	60
10	GPCR3 (SEQ ID NO:9)	GNITITLIVITQDSSLR	HEMFYEALISIDPGLST	TIKRMGTFWFT	LREISFE		
	Consensus 7tm 1 domain	GNITITLIVITQDSSLR	HEMFYEALISIDPGLST	TIKRMGTFWFT	LREISFE		
		70	80	90	100	110	120
15	GPCR3	GCLTOMFFIHTCTGMSAIVVAYPCVVAICDELCTLELNNVVSVMALAEITRPTF					
	Consensus 7tm 1 domain	GCLTOMFFIHTCTGMSAIVVAYPCVVAICDELCTLELNNVVSVMALAEITRPTF					
		130	140	150	160	170	180
20	GPCR3	VIRFVLEI--LPLPFCGR	QIIPHTYGEHNGEARLSCASR	VNIYIGL			
	Consensus 7tm 1 domain	VIRFVLEI--LPLPFCGR	QIIPHTYGEHNGEARLSCASR	VNIYIGL			
		190	200	210	220	230	240
25	GPCR3	CASILIFDNTAIVTSVDTIC					
	Consensus 7tm 1 domain	CASILIFDNTAIVTSVDTIC					
		250	260	270	280	290	300
30	GPCR3						
	Consensus 7tm 1 domain						
		310	320	330	340	350	360
35	GPCR3	AVELLSSHDAE--FAFSTCGS	HVCVMITF	MEAFFSFNTHRFRGN			
	Consensus 7tm 1 domain	AVELLSSHDAE--FAFSTCGS	HVCVMITF	MEAFFSFNTHRFRGN			
		370	380	390			
40	GPCR3	DEHFFHLLDANFYVVIIPALNSVTE					
	Consensus 7tm 1 domain	DEHFFHLLDANFYVVIIPALNSVTE					

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor-like protein may be useful in gene therapy, and the olfactory receptor-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from infections such as bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma,

Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. Other GPCR-related diseases and disorders are contemplated.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. The disclosed GPCR3 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR3 epitope is from about amino acids 160 to 190. In another embodiment, a GPCR3 epitope is from about amino acids 260 to 270. In an additional embodiment, GPCR3 epitopes are from amino acids 280 to 300.

#### **GPCR4**

GPCR4 includes a family of three nucleic acids disclosed below. The disclosed nucleic acids encode a GPCR-like protein.

##### **GPCR4a**

The disclosed GPCR4a (also referred to herein as AC020597\_B) is encoded by a nucleic acid, 994 nucleotides long (SEQ ID NO:10). An open reading frame was identified beginning with an ATG initiation codon at nucleotides 23-25 and ending with a TAA codon at nucleotides 968-970. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4A, and the start and stop codons are in bold letters. The encoded protein having 315 amino acid residues is presented using the one-letter code in Table 4B (SEQ ID NO:11).

**Table 4A. GPCR4a Nucleotide Sequence (SEQ ID NO:10).**

```

TGCTGAATTACTCAAAGTCACTATGGGAGACTGGAATAACAGTGATGCTGTGGAGCCCATATTTATCCTGAGGGG
TTTTCTGGACTGGAGTATGTTCACTTCTGGCTCTCCATCCTCTTCTGTCTTGCATATTGGTAGCATTTATGGG
TAATGTTACCATCCTGTCTGTCATTTGGATAGAATCCTCTCTCCATCAGCCCATGTATTACTTTATTTCCATCTT
AGCAGTGAATGACCTGGGATGTCCCTGTCTACACTTCCCACCATGCTTGTGTATGGTTGGATGCTCCAGA
GATCCAGGCAAGTGCTTGTATGCTCAGCTGTTCTTCATCCACACATTACATTCTGGAGTCCTCAGTGTGCT
GGCCATGGCCTTTGACCGTTTTGTGTATCTGCCATCCACTGCACTACCCACCATCCTCACCAACAGTGTAAAT
TGGCAAAATTTGGTTTGGCCTGTTGTGTACGAAGCTTGGGAGTTGTACTTCCCACACCTTTGCTACTGAGACACTA
TCACTACTGCCATGGCAATGCCCTCTCTCACGCCTTCTGTTGCACCAGGATGTTCTAAGATTATCCTGTACAGA
TGCCAGGACCAACAGTATTTATGGGCTTTGTGTAGTCATTGCCACACTAGGTGTGGATTCAATCTTCATACTTCT
TTCTTATGTTCTGATTCTTAATACTGTGCTGGATATTGCATCTCGTGAAGAGCAGCTAAAGGCACTCAACACATG
TGATATCCCATATCTGTGTGGTGTCTTCTTTGTGCCAGTTATTGGGGTGTCAATGGTCCATCGCTTTGGGAA
GCATCTGTCTCCCATAGTCCACATCCTCATGGCAGACATCTACCTTCTTCTTCCCCAGTCCTTAACCCATTGT
CTATAGTGTGAGAACAAAGCAGATTGCTTAGGAATTCTCCACAAGTTTGTCTAAGGAGGAGGTTTTAAGTAAC
CTCTGTCTCCAACCTTTTC

```

The disclosed nucleic acid GPCR4a sequence has 571 of 868 bases (65%) identical to a *Rattus norvegicus* GPCR mRNA (GENBANK-ID: AF079864).

- 5 The GPCR4a polypeptide (SEQ ID NO:11) encoded by SEQ ID NO:10 is presented using the one-letter amino acid code in Table 4B. The Psort profile for GPCR4a predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The most likely cleavage site for a GPCR4a peptide is between amino acids 40 and 41, at: LVA-FM., based on the SignalP result. GPCR4a has a molecular weight
- 10 of 35368.7.

**Table 4B. GPCR4a protein sequence (SEQ ID NO:11)**

```

MGDWNNSDAVEPIFILRGFPGLLEYVHWSLSILFCLAYLVAFMGNVTILSVIWIESSLHQPMMYFISILA
VNDLGMSLSTLPTMLAVLWLDAPETQASACYAQLFFIHTFTFLESSVLLAMAFDRFVAICHPLHYPTIL
TNSVIGKIGLACLLRSLGVVLPPLLLRHYHYCHGNALSHAFCLHQDVLRLSCTDARTNSIYGLCVVIA
TLGVDSIFILLSYVLIILNTVLDIASREEQLKALNTCVSHICVVLIFVFPVIGVSMVHRFGKHLSPIVHI
LMADIYLLLPVLPNPIVYSVRTKQIRLGILHKFVLRREF

```

- The full amino acid sequence of the disclosed GPCR4a polypeptide has 159 of 301 amino acid residues (52%) identical to, and 217 of 301 residues (72%) positive with, the 319 amino acid residue protein from *Gallus gallus* (ptnr:SPTREMBL-ACC: Q9YH55), ( $E = 1.0 \times 10^{-84}$ )
- 15

BLASTP (Non-Redundant Composite database) analysis of the best hits for alignments with GPCR4a are listed in Table 4C. BLASTX analysis was also performed to determine which proteins have significant identity with GPCR4a, as shown in Table 4D.

20

**Table 4C. BLASTP results for GPCR4a**

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ACC:Q9WVN4	MOR 5'BETA1 - Mus musculus	311	160/303 (52%)	219/303 (72%)	1.6e-83
ACC:O88628	PUTATIVE G-PROTEIN COUPLED RECEPTOR RA1C - Rattus norvegicus	320	152/296 (51%)	204/296 (68%)	5.3e-83
ACC:Q9Y5P1	HOR 5'BETA3 - Homo sapiens	312	145/312 (46%)	221/312 (70%)	1.9e-78

**Table 4D. BLASTX results for GPCR4a**

Table 4D. BLASTX results for GPCR4a					
Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N	
Ptnr:SPTREMBL-ACC:Q9YH55 OLFACTORY RECEPTOR-LIKE PROTE.+2		856	1.2e-84	1	
ptnr:SPTREMBL-ACC:Q9WVN4 MOR 5'BETA1 - Mus musculus (M.+2		838	9.5e-83	1	
ptnr:SPTREMBL-ACC:O88628 PUTATIVE G-PROTEIN COUPLED RE.+2		833	3.2e-82	1	
ptnr:SPTREMBL-ACC:Q9WVN5 MOR 5'BETA2 - Mus musculus (M.+2		830	6.7e-82	1	
ptnr:SPTREMBL-ACC:Q9WVN6 MOR 5'BETA3 - Mus musculus (M.+2		826	1.8e-81	1	
ptnr:SPTREMBL-ACC:Q9Y5P1 HOR 5'BETA3 - Homo sapiens (H.+2		790	1.2e-77	1	
ptnr:SPTREMBL-ACC:Q9WU90 ODORANT RECEPTOR S19 - Mus mu.+2		764	6.6e-75	1	
patp:Y92365 G protein-coupled receptor protein 5 - Hom +2		829	6.5e-82	1	
patp:W01730 Human G-protein receptor HPRAJ70 - Homo sa +2		818	9.6e-81	1	
patp:W56641 G-protein coupled prostate tissue receptor +2		818	9.6e-81	1	
patp:R27874 Odorant receptor clone I9 - Rattus rattus, +2		440	1.1e-40	1	
patp:R27875 Odorant receptor clone I14 - Rattus rattus +2		440	1.1e-40	1	

Possible SNPs found for GPCR4a are listed in Table4E.

**Table 4E: SNPs**

Base Position	Base Before	Base After
115	A	G(4)
140	G	gap(2)
141	C	gap(2)
174	A	gap(17)
175	C	gap(17)
176	A	gap(6)
177	C	gap(6)
178	A	gap(5)
179	C	gap(5)
180	gap	A(22)
181	gap	C(22)
182	gap	A(21)
183	C	gap(20)
185	C	gap(10)
229	gap	A(2)
252	A	gap(2)
256	gap	A(2)
548	T	C(2)

553	G	gap(2)
614	G	A(2)
1445	C	A(2)
1446	C	G(2)
1447	A	G(2)
1448	A	C(2)
1453	G	A(2)
1454	G	T(2)
1455	A	G(2)
1456	C	T(2)
1460	G	C(2)
1462	G	T(2)

### GPCR4b

The disclosed GPCR4b (also referred to herein as AC020597B1) is encoded by a nucleic acid, 994 nucleotides long (SEQ ID NO:12). An open reading frame was identified beginning with an ATG initiation codon at nucleotides 23-25 and ending with a TAA codon at nucleotides 968-970. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4F, and the start and stop codons are in bold letters. The encoded protein having 312 amino acid residues is presented using the one-letter code in Table 4G (SEQ ID NO:13).

10

**Table 4F. GPCR4b Nucleotide Sequence (SEQ ID NO:12)**

<u>TGCTGAATTACTCAAAGTCACTATGGGAGACTGGAATAACAGT</u> <u>GATGCTGTGGAGCCCATATTTATC</u> CTGAGGGGTTTTCTGGACTGGAGTATGTTCAATCTTGGCTCTCCATCCTCTTCTGTCTGCATATT TGGTAGCATTATGGGTAATGTTACCATCCTGTCTGTCAATTGGATAGAATCCTCTCTCCATCAGCC CATGTATTACTTTTATTTCCATCTTGGCAGTGAATGACCTGGGGATGTCCCTGTCTACACTTCCACC ATGCTTGCTGTGTTATGGTGGATGCTCCAGAGATCCAGGCAAGTGCTTGCTATGCTCAGCTGTTCT TCATCCACACATTACATTCTTGAGTCTCAGTGTGCTGGCCATGGCCTTTGACCGTTTGTGTC TATCTGCCATCCACTGCACCTACCCACCATCTCACCACAGTGTAATTGGCAAAATTGGTTGGCC TGTTTGCTACGAAGCTTGGGAGTTGTACTTCCACACCTTTGCTACTGAGACACTATCACTACTGCC ATGGCAATGCCCTCTCTCAGCCTTCTGTTGCACAGGATGTTCTAAGATTATCCTGTACAGATGC CAGGACCAACAGTATTTATGGGCTTTGTGTAGTCATTGCCACACTAGGFGTGGATTCAATCTTCATA CTTCTTTCTTATGTTCTGATTCTTAATACTGTGCTGGATATTGCATCTCGTGAAGAGCAGCTAAAGG CACTCAACACATGTGTATCCATATCTGTGTGGTGCTTATCTTCTTTGTGCCAGTTATTGGGGTGTC AATGGTCCATCGCTTGGGAAGCATCTGTCTCCATAGTCCACATCCTCATGGCAGACATGTACCTT CTTCTTCCCCAGTCCTTAACCTATTGTCTATAGTGTGAGAACAAAGCAGATTGCTCTAGGAATTC TCCACAAGTTTGTCTAAGGAGGAGGTTTAAAGTAACCTCTGTCTCCAACCTTTTC
---

The SignalP, Psort and/or Hydropathy profile for the disclosed GPCR4b Olfactory Receptor-like protein predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The SignalP shows a signal sequence is coded for in the first 36 amino acids with a cleavage site at between amino acids 40 and 41, between LVA/FM in Table 4G. The molecular weight of GPCR4b is 35386.7 Da. This is typical of this type of membrane protein.

15

**Table 4G. GPCR4b Amino Acid Sequence (SEQ ID NO:13)**

MGDWNNSDAVEPIFILRGFPGLEYVHSWLSILFCLAYLVAFMGNVTILSVIWISSLHQPMYFISILAVNDL  
 GMSLSTLPTMLAVLWDAPEIQASACYAQLFFIHTFTFLESSVLLAMAFDRFVAICHPLHYPTILTNSVIGKI  
 GLACLLRSLGVLPTPLLLRHHYCHGNALSHAFCLHQDVLRLSCTDARTNSIYGLCVVIATLGVDISIFILLS  
 YVLIINTVLDIASREEQLKALNTCVSHICVVLIFVFPVIGVSMVHRFGKHLSPIVHILMADMYLLLPVLPNPI  
 VYSVRTKQIRLGLHKEFVLRRLF

BLASTP alignments also showed high homology between GPCR4b and other proteins as shown in Table 4H.

**Table 4H. BLASTP results for GPCR4b**

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
SPTREMBL- ACC:Q9YH55	OLFACTORY RECEPTOR- LIKE PROTEIN COR3'BETA - Gallus gallus (Chicken),	319	159/301 (52%)	217/301 (72%)	2.7e-85
SPTREMBL- ACC:Q9WVN4	MOR 5'BETA1 - Mus musculus (Mouse)	311	160/303 (52%)	219/303 (72%)	2.2e-83
ACC:Q9Y5P1	HOR 5'BETA3 - Homo sapiens (Human)	312	144/312 (46%)	221/312 (70%)	3.4e-78

5

The disclosed GPCR4b protein is homologous to a number of olfactory receptor proteins, as shown in Table 4I.

**Table 4I. BLASTX results for GPCR4b**

Sequences producing High-scoring Segment Pairs:			Reading Frame	High Score	Smallest Sum Prob P(N)	N
ptnr:SPTREMBL-ACC:Q9YH55	OLFACTORY RECEPTOR-LIKE PROTE.	+2		854	1.6e-84	1
ptnr:SPTREMBL-ACC:Q9WVN4	MOR 5'BETA1 - Mus musculus (M	+2		836	1.3e-82	1
ptnr:SPTREMBL-ACC:O88628	PUTATIVE G-PROTEIN COUPLED RE.	+2		831	4.5e-82	1
ptnr:SPTREMBL-ACC:Q9WVN5	MOR 5'BETA2 - Mus musculus (M.	+2		828	9.3e-82	1
ptnr:SPTREMBL-ACC:Q9WVN6	MOR 5'BETA3 - Mus musculus (M.	+2		823	3.1e-81	1
ptnr:SPTREMBL-ACC:Q9Y5P1	HOR 5'BETA3 - Homo sapiens (H.	+2		787	2.1e-77	1
ptnr:SPTREMBL-ACC:Q9WU90	ODORANT RECEPTOR S19 - Mus mu.	+2		760	1.5e-74	1
ptnr:SPTREMBL-ACC:Q9WVD9	MOR 3'BETA1 - Mus musculus (M.	+2		751	1.3e-73	1
ptnr:SPTREMBL-ACC:Q9WU89	ODORANT RECEPTOR S18 - Mus mu.	+2		749	2.2e-73	1
ptnr:SPTREMBL-ACC:Q9Y5P0	HOR 5'BETA1 - Homo sapiens (H.	+2		745	5.8e-73	1
ptnr:SPTREMBL-ACC:Q9WVD7	MOR 3'BETA3 - Mus musculus (M.	+2		710	3.0e-69	1
ptnr:SPTREMBL-ACC:Q9WVD8	MOR 3'BETA2 - Mus musculus (M.	+2		705	1.0e-68	1
ptnr:SPTREMBL-ACC:Q9WU93	ODORANT RECEPTOR S46 - Mus mu.	+2		703	1.6e-68	1
ptnr:TREMBLNEW-ACC:CAB89291	OLFACTORY RECEPTOR - Homo	+2		532	1.8e-54	2

**GPCR4c**

10

The disclosed GPCR4c (also referred to herein as AC020597B2) is encoded by a nucleic acid, 994 nucleotides long (SEQ ID NO:14. An open reading frame was identified beginning with an ATG initiation codon at nucleotides 23-25 and ending with a TAA codon at nucleotides 968-970. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 4J, and the start and stop

codons are in bold letters. The encoded protein having 312 amino acid residues is presented using the one-letter code in Table 4J (SEQ ID NO:12).

Table 4J. GPCR4c Nucleotide Sequence (SEQ ID NO:14).	
<p> <b>TGCTGAATTACTCAAAGTCACTATGGGAGACTGGAATAACAGTGATGCTGTGGAGCCCATATTTATCCTGAGGGG</b>  <b>TTTTCTGGACTGGAGTATGTTCACTCTGGCTCTCCATCCTCTTCTGTCTTGATATTTGGTAGCATTTATGGG</b>  <b>TAAATGTTACCATCCTGTCTGTCAATTTGGATAGAATCCTCTCTCCATCAGCCCATGTATTACTTTATTTCCATCTT</b>  <b>GGCAGTGAATGACCTGGGGATGTCCTGTCTACACTTCCCACCATGCTTGTCTGTGTTATGGTTGGATGCTCCAGA</b>  <b>GATCCAGGCAAGTGCTTGTATGCTCAGCTGTTCTTCATCCACACATTCAATTCCTGGAGTCTCAGTGTGCT</b>  <b>GGCCATGGCCTTTGACCGTTTGTGCTATCTGCCATCCACTGCACTACCCACCATCCTCACCAACAGTGTAAAT</b>  <b>TGGCAAAATGGTTTGGCCTGTTGCTACGAAGCTTGGGAGTTGTAATTCACACACCTTGGCTACTGAGACACTA</b>  <b>TCATACTGCCATGGCAATGCCCTCTCTCACGCCTTCTGTTTGACCAGGATGTTCTAAGATTATCCTGTACAGA</b>  <b>TGCCAGGACCAACAGTATTTATGGGCTTGTGTAGTCATTGCCACACTAGGTGTGGATTCAATCTTCATACTTCT</b>  <b>TTCTTATGTTCTGATTCTTAATACTGTCTGGATATTGCATCTCGTGAAGAGCAGCTAAAGGCACTCAACACATG</b>  <b>TGTATCCCATATCTGTGTGGTGCTTATCTTCTTTGTGCCAGTTATGGGGTGTCAATGGTCCATCGCTTTGGGAA</b>  <b>GCATCTGTCTCCATAGTCCACATCCTCATGGCAGACATCTACCTTCTTCTCCCCAGTCCCTAACCCCTATTGT</b>  <b>CTATAGTGTGAGAACAAAGCAGATTCTGTCTAGGAATCTCCACAAGTTTGTCTTAAGGAGGAGGTTTTAAGTAAC</b>  <b>CTCTGTCTCCAACTTTTC</b> </p>	

The encoded protein is the same as for GPCR4a and is disclosed above in Table 4B.

5 Unless specifically addressed as GPCR4a or GPCR4b, any reference to GPCR4 is assumed to encompass all variants. Residue differences between any GPCR4 variant sequences herein are written to show the residue in the "a" variant and the residue position with respect to the "a" variant. In all following sequence alignments, the GPCR4a protein sequence was used.

10 The disclosed GPCR4 protein (SEQ ID NO:12) also has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 4K.

Table 4K. BLAST results for GPCR4					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 11908220 gb AAG41 684.1  (AF133300)	MOR 3'Beta4 [Mus musculus]	303	174/307 (56%)	223/307 (71%)	2e-81
gi 11908225 gb AAG41 688.1  (AF071080)	Mor 5'Beta5 [Mus musculus]	317	160/294 (54%)	215/294 (72%)	3e-79
gi 11908214 gb AAG41 679.1  (AF137396)	HOR5'Beta11 [Homo sapiens]	314	152/294 (51%)	226/294 (76%)	9e-79
gi 11908213 gb AAG41 678.1  (AF137396)	HOR5'Beta12 [Homo sapiens]	312	160/296 (54%)	211/296 (71%)	2e-78
gi 11908218 gb AAG41 683.1  (AF137396)	HOR5'Beta5 [Homo sapiens]	312	160/300 (53%)	220/300 (73%)	2e-77

15 This information is presented graphically in the multiple sequence alignment given in Table 4L (with GPCR4 being shown on line 1) as a ClustalW analysis comparing GPCR4 with related OR sequences.



Table 4L Information for the ClustalW proteins:

- 1) GPCR4A (SEQ ID NO:11)  
 2) GPCR4B (SEQ ID NO:13)  
 3) >gi|11908220|gb|AAG41684.1| (AF133300) MOR 3'Beta4 [Mus musculus] (SEQ ID NO:58)  
 4) >gi|11908225|gb|AAG41688.1| (AF071080) Mor 5'Beta5 [Mus musculus]gi|482652|emb|CAB42853.1| dJ8J8.1 (novel 7 transmembrane receptor (rhodopsin family) (OR like) pro (SEQ ID NO:59)  
 5) >gi|11908214|gb|AAG41679.1| (AF137396) HOR5'Beta11 [Homo sapiens]tein) (hs6M1-15)) Homo sapiens (SEQ ID NO:60)  
 6) >gi|11908213|gb|AAG41678.1| (AF137396) HOR5'Beta12 [Homo sapiens]gi|12054431|emb|CAC20523.1| olfactory receptor Homo sapiens (SEQ ID NO:61)  
 7) >gi|11908218|gb|AAG41683.1| (AF137396) HOR5'Beta5 [Homo sapiens]gi|12054429|emb|CAC20522.1| olfactory receptor Homo sapiens (SEQ ID NO:62)

10	20	30	40	50	60
GPCR4a	GDWNNDAVEPIETLRGPGLEYVHSNTSLFCHADVAFMGNVTILSFWIETSSLEH				
GPCR4b	GDWNNDAVEPIETLRGPGLEYVHSNTSLFCHADVAFMGNVTILSFWIETSSLEH				
gi 11908220 gb AAG41684.1	MATSNSTIVSSTFYLTCGPGYEEHHNHSFFCFLLVGHGNCMLHRTTPRLH				
gi 11908225 gb AAG41688.1	MKUSIPPRANFSYATPLTCTGPGLEWASHNHSPIFYGVAVMGNATILHRTTPSLH				
gi 11908214 gb AAG41679.1	HLGLNGPFPQATLQLTCTGPGYGTGLTWVAHIFCYLMTSTVGNLSLTTPWFPAPLH				
gi 11908213 gb AAG41678.1	MGLFVTHPAFELTCTGPGLESSHSNLSGPLCYMAVAFGGNVILQAVRVEPSLH				
gi 11908218 gb AAG41683.1	MSESSSSHPFLTCTGPGLEAHHNHSFFLFMTSILFNGTLLALIKEDHNLH				
70	80	90	100	110	120
GPCR4a	CPMYFFSTILAVNDLGMSLSTLPTEAVLNLPEPFOASACYAQFFIHTFFLESSVLL				
GPCR4b	CPMYFFSTILAVNDLGMSLSTLPTEAVLNLPEPFOASACYAQFFIHTFFLESSVLL				
gi 11908220 gb AAG41684.1	CPMYFFDAMLSITDMANSHPTMMSETRVLSISRETOFNICVVMELIHTFFLESSVLL				
gi 11908225 gb AAG41688.1	CPMYFFAILAVTDLGCMSTLPSYLCVLFEDARMYGLVECVLCOEHEHFSFMSAVLF				
gi 11908214 gb AAG41679.1	CPMYFFSMLAVNDLGVSFSTLPTESTFCFYNHAFNAQVCFIHTFFSMESGILL				
gi 11908213 gb AAG41678.1	CPMYFFSMLSFSPVANSMTALPTVETRTCLERNITFDACLTCMLIHTFFSMBSGILL				
gi 11908218 gb AAG41683.1	CPMYFFLAMLATDLGALTMPTVLCVLLHREHGSACFSKAFIHTFSELESGLLL				
130	140	150	160	170	180
GPCR4a	AMADDRVAICHPELHTPTLTSNVIGKIGLACLLRSLGVNLPPLLRHHHTCHGNALSH				
GPCR4b	AMADDRVAICHPELHTPTLTSNVIGKIGLACLLRSLGVNLPPLLRHHHTCHGNALSH				
gi 11908220 gb AAG41684.1	AMADDRVAICHPELRYATILTPKLTARIGTAALRSSILHILIAIAFAFPFGCSHVLSH				
gi 11908225 gb AAG41688.1	AMADDRVAICHPELRYATVLTGPRVALIGTVLGRSAITAAPSLHLLTDECHPGALSH				
gi 11908214 gb AAG41679.1	AMSDRRVAICHPELRYATVLTGPRVALIGTVLGRSAITAAPSLHLLTDECHPGALSH				
gi 11908213 gb AAG41678.1	AMSDRRVAICHPELRYATVLTGPRVALIGTVLGRSAITAAPSLHLLTDECHPGALSH				
gi 11908218 gb AAG41683.1	AMADDRVAICHPELRYATVLTGPRVALIGTVLGRSAITAAPSLHLLTDECHPGALSH				
190	200	210	220	230	240
GPCR4a	AYCLHQDVLRISCDARTNSIYGLCVVATLSTFELLSYVLIINLVLDIASREEQLK				
GPCR4b	AYCLHQDVLRISCDARTNSIYGLCVVATLSTFELLSYVLIINLVLDIASREEQLK				
gi 11908220 gb AAG41684.1	AYCLHQDMIRLACADIRENVLYGLVATLTLWGMSFGIFMSYVLIHHSVLRKIASREGRK				
gi 11908225 gb AAG41688.1	AYCLHQDMIRLACADIRENVLYGLVATLTLWGMSFGIFMSYVLIHHSVLRKIASREGRK				
gi 11908214 gb AAG41679.1	AYCLHQDMIRLACADIRENVLYGLVATLTLWGMSFGIFMSYVLIHHSVLRKIASREGRK				
gi 11908213 gb AAG41678.1	AYCLHQDMIRLACADIRENVLYGLVATLTLWGMSFGIFMSYVLIHHSVLRKIASREGRK				
gi 11908218 gb AAG41683.1	AYCLHQDMIRLACADIRENVLYGLVATLTLWGMSFGIFMSYVLIHHSVLRKIASREGRK				
250	260	270	280	290	300
GPCR4a	ALNTCVSHICVVLIFEVVIGVSMVHREFGKHLSEIVHILNADTYLLPPVLNPIVSVKT				
GPCR4b	ALNTCVSHICVVLIFEVVIGVSMVHREFGKHLSEIVHILNADTYLLPPVLNPIVSVKT				
gi 11908220 gb AAG41684.1	ALNTCVSHICVVLIFEVVIGVSMVHREFGKHLSEIVHILNADTYLLPPVLNPIVSVKT				
gi 11908225 gb AAG41688.1	ALNTCVSHICVVLIFEVVIGVSMVHREFGKHLSEIVHILNADTYLLPPVLNPIVSVKT				
gi 11908214 gb AAG41679.1	ALNTCVSHICVVLIFEVVIGVSMVHREFGKHLSEIVHILNADTYLLPPVLNPIVSVKT				
gi 11908213 gb AAG41678.1	ALNTCVSHICVVLIFEVVIGVSMVHREFGKHLSEIVHILNADTYLLPPVLNPIVSVKT				
gi 11908218 gb AAG41683.1	ALNTCVSHICVVLIFEVVIGVSMVHREFGKHLSEIVHILNADTYLLPPVLNPIVSVKT				
310	320				
GPCR4a	KQIRLGILHREVLRRF----				
GPCR4b	KQIRLGILHREVLRRF----				
gi 11908220 gb AAG41684.1	KQIRRGILHLLCSPEISSITM				
gi 11908225 gb AAG41688.1	KQIRRAVKKVLSLGHQ----				
gi 11908214 gb AAG41679.1	KQIRKGIKKFHKSOA----				
gi 11908213 gb AAG41678.1	KQIRRAVKKVLSLGHQ----				
gi 11908218 gb AAG41683.1	KQIRRAVKKVLSLGHQ----				

DOMAIN results for GPCR4 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. Two regions of GPCR4 have identity to the 377 amino acid 7TM domain, as described above. The 7tm\_1, a seven transmembrane receptor (rhodopsin family), was shown to have homology to GPCR4 ( $E = 4e-17$ ) (Table 4M).

**Table 4M: Domain Alignment between GPCR4 and 7tm\_1, 7 transmembrane receptor (rhodopsin family) (SEQ ID NO:74).**

CD-Length = 254 residues, 100.0% aligned  
Score = 82.0 bits (201), Expect = 4e-17

		10	20	30	40	50	60
GPCR4		.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain		GNVTILSVIWIESSLH-QEYFESILAVNDLGMSLSTLPTMLAVLWDAPEIQASAYAGNELHLVILRTKKLR-TETNIFVLNLAVADILFLTLPLPWATYYLVGGDWVFGDILCKL					
		70	80	90	100	110	120
GPCR4		.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain		QLFFIHTFTFLESSVLLAMDFDRVAICHPLHYPTILTNSVIGKIGLACLRSELCVIVETVGALFVVNGTASILETAKSIDFYLAIVHPURRIRIPRRAKVILLVWVLLALLESIFP					
		130	140	150	160	170	180
GPCR4		.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain		PULLRRHYHYCHGNA-----LSHAFCLHIVVSR--LSCDARTNSIVGLCVIATLGVDISIFLFSWLRTVEEGHT---TVCLIDFPFESVSR--RSYVLLSLGVGFLPPLVLELVICYTR					
		190	200	210	220	230	240
GPCR4		.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain		TLISYVLILN-----LIRRLRSAR-----					
		250	260	270	280	290	300
GPCR4		.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain		-----IVLDIASPE -----SORSKKRFS					
		310	320	330	340	350	360
GPCR4		.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain		E-ILKALNTCPSEHCVVHFEVIGVSMVHRFGKHLSP-----VHILMADIVYLLTSSERKAAMLVVVVVFVICKLIPYHIVLLDLSCLLSIWR-----VLPTALLITLML					
		370					
GPCR4		.....					
Consensus 7tm 1 domain		P---PVLNPIVY AYVNSCLNPTILN					

An alignment of GPCR4a and b amino acid sequences is shown in Table 4N.

**Table 4N. Alignment between GPCR4a and b.**

		10	20	30	40	50	60
GPCR4a		.....	.....	.....	.....	.....	.....
GPCR4b		MGDWNNSDAVEPIFILRGFPGLGYVHSWLSILFCLAYLVAFMGNVTILSVIWIESSLHQP					
		70	80	90	100	110	120
GPCR4a		.....	.....	.....	.....	.....	.....
GPCR4b		MYFISILAVNDLGMSLSTLPTMLAVLWDAPEIQASACYAQLFFIHTFTFLESSVLLAM					
		130	140	150	160	170	180
GPCR4a		.....	.....	.....	.....	.....	.....
GPCR4b		AFDRFVAICHPLHYPTILTNSVIGKIGLACLLRSLGVVLPPTPLLLRHYHYCHGNALSHAF					
		190	200	210	220	230	240
GPCR4a		.....	.....	.....	.....	.....	.....
GPCR4b		CLHQDVLRLSCTDARTNSIYGLCVVIATLGVDISIFILLSYVLILNTVLDIASREEQLKAL					

		250	260	270	280	290	300
5	GPCR4a	NTCVSHICVVLIF	FEVPVIGVSMVHRFGKHLSP	IVHILMADNYLLLP	PVLNPIVYSVRTK		
	GPCR4b	NTCVSHICVVLIF	FEVPVIGVSMVHRFGKHLSP	IVHILMADNYLLLP	PVLNPIVYSVRTK		
		310					
10	GPCR4a	IRLGILHKFVLR	RRF				
	GPCR4b	IRLGILHKFVLR	RRF				

The nucleic acids and proteins of GPCR4 are useful in potential therapeutic applications implicated in various GPCR-related pathological disorders and/or OR-related pathological disorders, described further below. For example, a cDNA encoding the olfactory receptor-like protein may be useful in gene therapy, and the olfactory receptor-like protein may be useful when administered to a subject in need thereof. The protein similarity information, expression pattern, and map location for the Olfactory Receptor-like protein and nucleic acid disclosed herein suggest that this Olfactory Receptor may have important structural and/or physiological functions characteristic of the Olfactory Receptor family.

Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon.

The nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from: bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette and/or other pathologies and disorders of the like. The polypeptides can be used as immunogens to produce antibodies

specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the OR -like protein may be useful in gene therapy, and the OR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome. Other GPCR-4 diseases and disorders are contemplated.

The novel nucleic acid encoding the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. The disclosed GPCR4 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR4 epitope is from about amino acids 160 to 200. In another embodiment, a GPCR4 epitope is from about amino acids 230 to 250. In an additional embodiment, GPCR4 epitopes are from amino acids 290 to 305.

## **GPCR5**

GPCR5 includes a family of three similar nucleic acids and three similar proteins disclosed below. The disclosed nucleic acids encode GPCR, OR-like proteins.

### **GPCR5a**

The disclosed novel GPCR5a nucleic acid of 985 nucleotides (also referred to as 1 AC020597\_C) is shown in Table 5A. An ORF begins with an ATG initiation codon at nucleotides 27-29 and ends with a TAA codon at nucleotides 960-962. A putative

untranslated region upstream from the initiation codon and downstream from the termination codon is underlined in Table 5A, and the start and stop codons are in bold letters.

**Table 5A. GPCR5a Nucleotide Sequence (SEQ ID NO:15)**

**GT**TCTCCTACACTGTGATTGGAAAAATGTTTATCACAACAAGAGCATATTTACCCAGTCACATTTTCTCA  
 TTGGAATCCAGGCTCTGGAAGACTTCCACATGTGGATCTCCGGGCTTTCTGCTCTGTTTACCTTGTGGCTTTGC  
 TGGGCAATGCCACCATTCTGCTAGTCATCAAGGTAGAACAGACTCTCCGGGAGCCCATGTTCTACTTCTGGCCA  
 TTCTTTCCACTATGATTGGCCCTTTCTGCAACCTCTGTGCCTCGCATGCTGGGTATCTTCTGGTTTGATGCTC  
 ACGAGATTAAGTATGGAGCTTGTGTGGCCAGATGTTCTGATCCATGCCTTCACTGGCATGGAGCTGAGGTCT  
 TACTGGCTATGGCTTTTGACCGTATGTGGCCATCTGTGCTCCACTACATTACGCAACCATCTTGACATCCCTAG  
 TGTGGTGGGCATTAGCATGTGCATTGTAATTCGTCCCGTTTACTTACACTTCCCATGGTCTATCTTATCTACC  
 GCCTACCCCTTTGTGAGGCTCACATAATAGCCCATTCCTACTGTGAGCACATGGGCATGCAAAATTGTCTGTG  
 GAAACATTGATCAATGGTATCTATGGGCTTTTGTAGTTTCTTCTTGTCTGAACCTGGTGCTCATTGGCA  
 TCTCGTATGTTACATTCTCCGTGCTGCTTCCGCCTCCCATCACATGATGCTCAGCTAAAAGCCCTAAGCACGT  
 GTGGCGCTCATGTTGGAGTCATCTGTGTTTCTATATCCCTTCAGTCTTCTTCTTCTTACTCATCGATTTGGAC  
 ACCAATACAGGTTACATTACATTCTGTTGCCAATCTCTATTGATTATCCACCCCTCTCTCAACCCCATCA  
 TTTATGGGGTGAGGACCAACAGATTGAGAGCGAGTGTCTATGTTTTACTAAAAATAAGACTCTTACCATG  
 TTATTTTACT

- 5 The GPCR5a protein encoded by SEQ ID NO:15 has 311 amino acid residues and is presented using the one-letter code in Table 5B. The Psort profile for GPCR5a predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The most likely cleavage site for a peptide is between amino acids 49 and 50: between ILL-VI based on the SignalP result. The molecular weight of GPCR5a is 35059.6 Daltons.

**Table 5B. Encoded GPCR5a protein sequence (SEQ ID NO:16)**

MFYHNKSI FHPVTFFLIGIPGLEDFHMMWISGPFCSVYLVALIGNATILLVIKVEQTLREPMFYFLAILSTIDL  
 ALSATSVPRMLGIFWFDAHEINYGACVAMFLIHAFTGMEAEVLLAMAFDRYVAICAPLHYATILTSVLVGI  
 SMCIVIRPVLTLPMVYLIYRLPFCQAHIIAHSYCEHMGIAKLSGNIIRINGIYGLFVVSFFVLNLVLIGISY  
 VYILRAVFRLP SHDAQLKALSTCGAHVGVICVFYIPSVFSFLTHRFGHQIPGYIHILVANLYLIIPPSLNPII  
 YGVRTKQIRERVLYVFTKK

The disclosed nucleic acid sequence for GPCR5a has 633 of 989 bases (64%) identical to a *Mus musculus* GPCR mRNA (GENBANK-ID: AF121979) ( $E = 7.2e^{-60}$ ).

- 15 The full GPCR5a amino acid sequence has 174 of 303 amino acid residues (57%) identical to, and 231 of 303 residues (76%) positive with, the 318 amino acid residue odorant receptor S46 protein from *Mus musculus* (ptnr:SPTREMBL-ACC: Q9WU93) ( $E = 1.9e^{-92}$ ) and 146 of 310 amino acid residues (47%) identical to, and 212 of 310 residues (68%) positive with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (ptnr: TREMBLNEW-ACC:AAD51279) ( $E = 2.1e^{-77}$ ).

GPCR5a also has homology to other proteins as shown in BLAST alignment results in Table 5C.

**Table 5C. BLASTX results for GPCR5a**

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
92365 G protein-coupled receptor protein 5 - Hom...	+3	778	1.7e-76	1
01730 Human G-protein receptor HPRAJ70 - Homo sa..	+3	735	6.0e-72	1
56641 G-protein coupled prostate tissue receptor...	+3	735	6.0e-72	1
27875 Odorant receptor clone I14 - Rattus rattus...	+3	460	8.3e-43	1
27874 Odorant receptor clone I9 - Rattus rattus,...	+3	438	1.8e-40	1

**GPCR5b**

GPCR5a (AC020597\_C) was subjected to an exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide GPCR5b, which is also referred to as CG53668-02.

The nucleotide sequence for GPCR5b (947 bp, SEQ ID NO:17) is presented in Table 5D.

**Table 5D. GPCR5b Nucleotide Sequence (SEQ ID NO:17)**

```

TGAAAAATGTTTTATCACAACAAGAGCATATTTACCCAGTCACATTTTTCCTCATTGGAATCCCAGGCTGGAA
GACTTCCACATGTGGATCTCCGGGCCTTTCTGCTCTGTTTACCTTGTGGCTTTGCTGGGCAATGCCACCATTCTG
CTAGTCATCAAGGTAGAACAGACTCTCCGGGAGCCCATGTTCTACTTCCGGCCATTCTTCCACTATTGATTG
GCCCTTTCTACAACCTCTGTGCCTCGCATGCTGGGTATCTTCTGGTTTGATGCTCAGAGATTAACTATGGAGCT
TGTGTGGCCAGATGTTTCTGATCCATGCCTTCACTGGCATGGAGGCTGAGGCTTACTGGCTATGGCTTTTGAC
CGTTATGTGGCCGCTGTGCTCCACTACATTACGCAACCATCTTGACATCCCAAGTGTTGGTGGGCATTAGCATG
TGCATTGTAATCCGTCCTGTTTACTTACACTTCCCATGGTCTATCTTATCTACCGCTACCCCTTTTGTGAGGCT
CACATAATAGCCCATCTTACTGTGAGCACATGGGCATTGCAAAATTGTCCTGTGGAACATTGATCAATGGT
ATCTATGGGCTTTTGTAGTTTCTTCTTTGTTCTGAACCTGGTGCTCATTGGCATCTCGTATGTTTACATTCTC
CGTGCTGTCTTCCGCTCCCATCACATGATGCTCAGCTAAAGCCCTAAGCACGTGTGGCGCTCATGTTGGAGTC
ATCTGTGTTTCTATATCCCTTCACTCTTCTTTCTTACTCATCGATTGGACACCAATAACAGGTTACATT
CACATTCTTGTGCAATCTCTATTTGATTATCCACCCTCTCTCAACCCCATCATTTATGGGGTGAGGACCAA
CAGATTCGAGAACGAGTGCTCTATGTTTACTAAAAAATAAGACTA

```

The encoded GPCR5b protein is presented in Table 5E. The disclosed protein is 311 amino acids long and is denoted by SEQ ID NO:18. GPCR5b differs from GPCR5a by 3 amino acid changes in positions 77 A->T, 128 I->V and 141 L->Q. Like GPCR5a, the Psort profile for GPCR5b predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The most likely cleavage site for

a peptide is between amino acids 49 and 50, *i.e.*, at the slash in the amino acid sequence ILL-VI based on the SignalP result.

**Table 5E. Encoded GPCR5b protein sequence (SEQ ID NO:18)**

```
MFYHNKSI FHPVTFFLIGIPGLEDFH MWISGPFCSVYL VALLGNATILLVIKVEQTLREPMFYFLAILSTIDLAL
STTSVFRMLGIFWFDAHEINYGACVAQMFLIHAFTGMEAEVLLAMAFDRYVAVCAPLHYATILTSQVLVGISMCI
VIRPVLLTLEPMVYLIYRLPFCQAHIIAHSYCEHMGIAKLSCGNIRINGIYGLFVVSFFVLNLVLIGISYVYILRA
VFRLP SHDAQLKALSTCGAHVGVICVFYIPSVFSLTHRFGHQIPGYIHILVANLYLIIPPSLNPIIYGVRTKQI
RERVLYVFTKK
```

The disclosed nucleic acid sequence for GPCR5b has 598 of 921 (64%) identical to a *Mus musculus* odorant receptor S46 mRNA (GENBANK-ID: AF121979) ( $E=9.5e^{-60}$ ).

The full GPCR5 amino acid sequence has 174 of 303 amino acid residues (57%) identical to, and 233 of 303 (76%) positive with, the 318 amino acid residue odorant receptor S46 protein from *Mus musculus* (ptnr:SPTREMBL-ACC: Q9WU93) ( $E=9.7e^{-92}$ ).

GPCR5b is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, MHC II and III expressing cells, nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources.

#### GPCR5c

Another nucleotide sequence resulted when GPCR5a (AC020597\_C) was subjected to an exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and

reverse primers in a PCR amplification based on a wide range of cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide the sequence reported below, which is designated as Accession Number AC020597B\_da1, or GPCR5c.

- 5 The nucleotide sequence for GPCR5c (945 bp, SEQ ID NO:19) is presented in Table 5F

Table 5F. GPCR5c Nucleotide Sequence (SEQ ID NO:19)	
GAAAAATGTTTATCACACAAGAGCATATTTACCCAGTCACATTTTCTCATTTGGAATCCCAGGTCT GGAAGACTTCCACATGTGGATCTCCGGGCCTTTCTGCTCTGTTTACCTTGCGGCTTTGCTGGGCAATGCC ACCATTCTGCTAGTCATCAAGGTAGAACAGACTCTCCGGGAGCCCATGTTCTACTTCTGGCCATTCTTT CCACATTATGATTTGGCCCTTTTACAACCTCTGTGCCTCGCATGCTGGGTATCTTCTGGTTTGATGCTCA CGAGATTAACATATGGAGCTTGTGTGGCCAGATGTTTCTGATCCATGCCTTCACTGGCATGGAGGCTGAG GTCTTACTGGCTATGGCTTTTGACCGTTATGTGGCCGCTGTGCTCCACTACATTACGCAACCATCTTGA CATCCCAAGTGTGGTGGGCATTAGCATGTGCATTGTAATTCGTCCCGTTTACTTACACTTCCCATGGT CTATCTTATCTACCGCCTACCCCTTTTGTGAGGCTCACATAATAGCCCATTCCTACTGTGAGCACATGGGC ATTGCAAAATTGTCCTGTGGAACATTTCGTATCAATGGTATCTATGGGCTTTTGTAGTTTCTTTCTTTG TTCTGAACCTGGTGCTCATTTGGCATCTCGTATGTTTACATTCTCCGTGCTGTCTTCCGCCTCCATCACA TGATGCTCAGCTAAAAGCCCTAAGCACGTGTGGCGCTCATGTTGGAGTCATCTGTGTTTCTATATCCCT TCAGTCTTCTCTTCTTACTCATCGATTGGACACCAAATACCAGGTTACATTACATTCTTGTGCGCA ATCTCTATTGATTATCCACCCCTCTCTCAACCCCATCATTTATGGGGTGAGGACCAACAGATTGAGA ACGAGTGCTCTATGTTTTTACTAAAAATAAGACT	

- 10 The coding region of GPCR5c is from nucleotide 6 to 938, giving the encoded GPCR5c protein, as presented in Table 5G. The disclosed protein is 311 amino acids long and is denoted by SEQ ID NO: 20. The Psort profile for GPCR5c predicts that this sequence has a signal peptide and is likely to be localized at the endoplasmic reticulum with a certainty of 0.6850 or plasma membrane with a certainty of 0.6400. The most likely cleavage site for a peptide is between amino acids 49 and 50, ILL-VI based on the SignalP result. The molecular weight of GPCR5c is 35062.6 Daltons.

Table 5G. Encoded GPCR5c protein sequence (SEQ ID NO:20)	
MFYHNKSI FHPVTF FLIGIP GLED FHMWIS GPFC SVYLA ALLGNAT ILLVIKVE QTLRE PMFY FLAILSTI DLALSTTSVP RMLGIFW FDAHEINYGACVAQM FLIHAFTGMEAEVLLAMAFDRYVAVCAPLHYATILTSQV LVGISMCIVIRPVL LTPMVYLIYRLPFCQAHIIAHSYCEHMGIAKLSCGNIRINGIYGLFVVSFFVLNLV LIGISYVYILRAVFR LPSHDAQLKALSTCGAHVGVICVFYIPSVFSFLTHRFHQIPGYIHILVANLYLII PPSLNPIIYGVRTKQIRERVLYVETKK	

- 20 The full GPCR5c amino acid sequence has 174 of 303 amino acid residues (57%) identical to, and 232 of 303 residues (76%) positive with, the 318 amino acid residue odorant receptor S46 protein from *Mus musculus* (ptnr:SPTREMBL-ACC: Q9WU93) ( $E=2.2e^{-92}$ ) and 146 of 310 amino acid residues (47%) identical to, and 212 of 310 residues (68%) positive



with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (ptr: SPTREMBL-ACC:Q9UKL2) ( $E = 2.1e^{-77}$ ).

Possible SNPs found for GPCR5c are listed in Table 5H.

Table 5H: SNPs		
Base Position	Base Before	Base After
63	T	C(3)
94	C	T(4)
110	C	T(4)
114	A	G(4)
157	G	T(2)
170	T	C(2)
197	G	C(2)
242	T	C(3)
262	G	A(2)
290	G	A(3)
299	G	C(3)
314	G	T(2)
316	A	C(2)
329	C	T(2)
332	A	C(3)
333	T	A(3)
356	A	C(3)
376	A	G(3)
377	C	T(2)
396	A	G(3)
428	C	T(2)
453	A	G(2)

5

The disclosed GPCR5 protein (SEQ ID NO:19) has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 5J. Unless specifically addressed as GPCR5a GPCR5b, or GPCR5c, any reference to GPCR5 is assumed to encompass all variants. All GPCR5 proteins have significant homology to olfactory receptor (OR) proteins: The homology information from BLASTX alignments for the proteins in the ClustalW is presented in Table 5I.

10

Table 5I. BLAST results for GPCR5

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 11908211 gb AAG41676.1  (AF137396)	HOR 5'Beta14 [Homo sapiens]	318	166/297 (55%)	217/297 (72%)	7e-81
gi 9935442 ref NP_064688.1	odorant receptor S46 gene [Mus musculus]	318	168/303 (55%)	218/303 (71%)	1e-80
gi 6532001 gb AAD27596.2 AF121976_1 (AF121976)	odorant receptor S19 [Mus musculus]	339	162/291 (55%)	208/291 (70%)	1e-79
gi 9938014 ref NP_064686.1	odorant receptor S18 gene [Mus musculus]	321	158/299 (52%)	218/299 (72%)	1e-79
gi 7305349 ref NP_038647.1	olfactory receptor 67 [Mus musculus]	326	164/309 (53%)	222/309 (71%)	5e-78

This information is presented graphically in the multiple sequence alignment given in Table 5J (with GPCR5a being shown on line 1 and GPCR5b on line 2) as a ClustalW analysis comparing GPCR5 with related protein sequences.

Table 5J Information for the ClustalW proteins:

- 1) GPCR5a (SEQ ID NO:16)
- 2) GPCR5b (SEQ ID NO:18)
- 3) gi|11908211|gb|AAG41676.1| (AF137396) HOR 5'Beta14 [Homo sapiens] (SEQ ID NO:55)
- 4) gi|9935442|ref|NP\_064688.1| odorant receptor S46 gene [Mus musculus] (SEQ ID NO:63)
- 5) gi|6532001|gb|AAD27596.2|AF121976\_1 (AF121976) odorant receptor S19 [Mus musculus] (SEQ ID NO:54)
- 6) gi|9938014|ref|NP\_064686.1| odorant receptor S18 gene [Mus musculus] (SEQ ID NO:56)
- 7) gi|7305349|ref|NP\_038647.1| olfactory receptor 67 [Mus musculus] (SEQ ID NO:57)

	10	20	30	40	50	60
GPCR5a	.....	.....	.....	.....	.....	.....
GPCR5b	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
	70	80	90	100	110	120
GPCR5a	.....	.....	.....	.....	.....	.....
GPCR5b	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
	130	140	150	160	170	180
GPCR5a	.....	.....	.....	.....	.....	.....
GPCR5b	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....	.....

gi 6532001	SYHCCHTQMFEYHAFVATSAVLLAMAFDRYVAICRPLHYTSLLNAVVGKIGLACTG
gi 9938014	PEDACTACMFETIHFVAFVDSGLLAMAFDRYVAICRPLRYSAULTPMAIGKMTLAIRGRS
gi 7305349	SEGSCTVSCMFETIHFVAFVDSGLLAMAFDRYVAICRPLRYVTTLTSSVIGKIGTAAVPS
	.....190.....200.....210.....220.....230.....240.....
GPCR5a	YVLTLPWYLYRIRLPFCQAHIIAHSYCEHMGIAKLSGCHIRNGHYGL-FVVSFFVNLV
GPCR5b	YVLTLPWYLYRIRLPFCQAHIIAHSYCEHMGIAKLSGCHIRNGHYGL-FVVSFFVNLV
gi 11908211	YVLTLPWYLYRIRLPFCQAHIIAHSYCEHMGIAKLSGCHIRNGHYGL-FVVSFFVNLV
gi 9935442	YVLTLPWYLYRIRLPFCQAHIIAHSYCEHMGIAKLSGCHIRNGHYGL-FVVSFFVNLV
gi 6532001	YVLTLPWYLYRIRLPFCQAHIIAHSYCEHMGIAKLSGCHIRNGHYGL-FVVSFFVNLV
gi 9938014	YVLTLPWYLYRIRLPFCQAHIIAHSYCEHMGIAKLSGCHIRNGHYGL-FVVSFFVNLV
gi 7305349	YVLTLPWYLYRIRLPFCQAHIIAHSYCEHMGIAKLSGCHIRNGHYGL-FVVSFFVNLV
	.....250.....260.....270.....280.....290.....300.....
GPCR5a	LIGISYVYILRAVERLPSHDAQKALSTCGAHVGVLCVFIYIPVFSFELTHREG-HQIECY
GPCR5b	LIGISYVYILRAVERLPSHDAQKALSTCGAHVGVLCVFIYIPVFSFELTHREG-HQIECY
gi 11908211	LIGISYVYILRAVERLPSHDAQKALSTCGAHVGVLCVFIYIPVFSFELTHREG-HQIECY
gi 9935442	LIGISYVYILRAVERLPSHDAQKALSTCGAHVGVLCVFIYIPVFSFELTHREG-HQIECY
gi 6532001	LIGISYVYILRAVERLPSHDAQKALSTCGAHVGVLCVFIYIPVFSFELTHREG-HQIECY
gi 9938014	LIGISYVYILRAVERLPSHDAQKALSTCGAHVGVLCVFIYIPVFSFELTHREG-HQIECY
gi 7305349	LIGISYVYILRAVERLPSHDAQKALSTCGAHVGVLCVFIYIPVFSFELTHREG-HQIECY
	.....310.....320.....330.....340.....
GPCR5a	YHILVANLYLIPESLNPIIYGVTQKQIDPFVYFFTKK-----
GPCR5b	YHILVANLYLIPESLNPIIYGVTQKQIDPFVYFFTKK-----
gi 11908211	YHILVANLYLIPESLNPIIYGVTQKQIDPFVYFFTKK-----
gi 9935442	YHILVANLYLIPESLNPIIYGVTQKQIDPFVYFFTKK-----
gi 6532001	YHILVANLYLIPESLNPIIYGVTQKQIDPFVYFFTKK-----
gi 9938014	YHILVANLYLIPESLNPIIYGVTQKQIDPFVYFFTKK-----
gi 7305349	YHILVANLYLIPESLNPIIYGVTQKQIDPFVYFFTKK-----

DOMAIN results for GPCR5 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 5K with the statistics and domain description.

**Table 5K. DOMAIN results for GPCR5 against the consensus 7tm 1 domain (SEQ ID NO:75)**

CD-Length = 254 residues, 100.0% aligned  
Score = 60.5 bits (145), Expect = 1e-10

		10	20	30	40	50	60
10	GPCR5 (SEQ ID NO:16)	GNATILLVIRKVEITL	EEPMFYFLAI	STIDLSA	SVSRM	GIFWFD	AAHEINYG
	Consensus 7tm 1 domain	GNLLLVIRLRTKKS	TTTNI	FLLN	AVADILFL	LPPWA	YYLVGGDWVFGDALC
		70	80	90	100	110	120
15	GPCR5	ACVAQMGIIHAFTGMEAEVLLAND	FDYVAICAPLHYATIL	SLVLVG	ESKCEVTRP	VILL	
	Consensus 7tm 1 domain	KLVGALSVNGYA	SSIL	LTATSI	DRYATVHPL	RYRRIR	PRRAKVHILVWLLALIL
		130	140	150	160	170	180
20	GPCR5	FLPMVYLI	YRL	PECD	AHIIA	SYC	EHVGTAKLSGCHIR
	Consensus 7tm 1 domain	SLPEPLFS		SLT	VEEGNT		TVCHIDFPEEBVS
		190	200	210	220	230	240
25	GPCR5	INGIYGLFVVSFFVNLV	LGTSYVYILRAVER				
	Consensus 7tm 1 domain	RSYVILSTVGGELPPLV	LLVCL	TRILP	TRFS	SARSORS	
		250	260	270	280	290	300

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involving photoreception, cell growth rate disorders, cell shape disorders, feeding disorders, potential obesity due to over-eating, potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, allergies, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease, multiple sclerosis, Albright hereditary osteodystrophy, angina pectoris, myocardial infarction, ulcers, benign prostatic hypertrophy, psychotic and neurological disorders (including anxiety, schizophrenia, manic depression, delirium, dementia, and severe mental retardation), dentatorubro-pallidoluysian atrophy (DRPLA), hypophosphatemic rickets, autosomal dominant (2) acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. For example the disclosed GPCR5 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR5 epitope is from about amino acids 170 to 200. In another embodiment, a GPCR5 epitope is from about amino acids 230 to 250. In additional embodiments, GPCR5 epitopes are from amino acids 270 to 310. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

## **GPCR6**

### **GPCR6a**

The disclosed novel GPCR6a nucleic acid of 1012 nucleotides (also referred to as AC020597\_D) is shown in Table 6A. An open reading begins with an ATG initiation codon at nucleotides 16-18 and ends with a TGA codon at nucleotides 991-993. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 6A, and the start and stop codons are in bold letters.

**Table 6A. GPCR6a Nucleotide Sequence (SEQ ID NO:21)**

```
GCATTACAAGCAGGATGTTCTTCCCAATGACACCCAGTTTACCCCTCCTCCTTCTGTTGCTGGGGATCCCA
GGACTAGAAACACTTCACATCTGGATCGGCTTCCCTTCTGTGCTGTGTACATGATCGCACTCATAGGAACTTC
ACTATTCTACTTGTGATCAAGACTGACAGCAGCCTACACCAGCCATGTTCTACTTCTGGCCATGTTGGCCACC
ACTGATGTGGGTCTCTCAACAGCTACCATCCCTAAGATGCTTGGAACTTCTGGATCAACCTCAGAGGGATCATC
TTTGAAGCCTGCCTCACCAGATGTTTTTATCCACAACCTTCACTTATGGAGTCAGCAGTCCTTGTGGCAATG
GCTTATGACAGCTATGTGGCCATCTGCAATCCAATATAGCGCCATCCTCACCACAAGGTTGTTTCTGTG
ATTGGTCTTGGTGTGTTTGTGAGGGCTTAAATTTTCGTTCATTCCTCTATACTTCTTATATTGCGGTGGCCCTTC
TGTGGGAATCATGTAATCCCCACACCTACTGTGAGCACATGGGTCTTGCTCATCTATCTTGTGCCAGCATCAA
ATCAATATTATTATGGTTTATGTGCCATTTGTAATCTGGTGTGTTGACATCACAGTCATTGCCCTCTCTTATGTG
CATATTCTTGTGCTGTTTCCGTCTTCTACTCATGAGCCCGACTCAAGTCCCTCAGCACATGTGTTTCACAT
GTGTGTGTAATCCTTGCCTTCTATACACCGCCCTCTTTTCTTATGACTCATTGCTTTGGCCGAATGTGCC
CGCTATATCCATATACTCTAGCCAATCTCTATGTTGTGGTGGCCACCAATGCTCAATCTGTATATATGGAGTC
AGAACCAAGCAGATCTATAAATGTGTAAGAAAATATTATTCAGGAACAAGGAATGGAAAAGGAAGAGTACCTA
ATACATACGAGGTTCTGAATGCAATTTATGAAATTT
```

The disclosed nucleic acid sequence has 662 of 1005 bases (65%) identical to a *Mus musculus* odorant receptor S46 mRNA (GENBANK-ID: AF121979) (E value =  $1.8e^{-70}$ ).

The GPCR6a protein encoded by SEQ ID NO:21 has 325 amino acid residues, and is presented using the one-letter code in Table 6B (SEQ ID NO:22). The Psort profile for GPCR6a predicts that this sequence has a signal peptide and is likely to be localized at the endoplasmic reticulum with a certainty of 0.6850 or plasma membrane with a certainty of 0.6400. The most likely cleavage site for a peptide is between amino acids 55 and 56, TDS-SL based on the SignalP result. The molecular weight of GPCR6a is 36602.5 Daltons.

**Table 6B. Encoded GPCR6a protein sequence (SEQ ID NO:22).**

```
MFLPNDTQFHPSSFLLLGIPGLETLHIWIGFFPCAVYMIALIGNFTILLVIKTDSSLHQPMFYFLAMLATTDVGL
STATIPKMLGIFWINLRGIIFEACLTQMFFIHNFTLMESAVLVAMAYDSYVAICNPLQYSAILTNKVVSIVIGLV
FVRALIFVIPSILLILRLPFCGNHVIPTHYCEHMGHLSCASIKINIIYGLCAICNLVFDITVIALSYVHILCA
VFRLPTHEPRLKSLSTCGSHVCVILAFYTPALFSFMTHCFGRNVPRYIHIILLANLYVVVPPMLNPVIYGVR
TKQIYKCVKKILLQEQGMEKEEYLIHTRF
```

The full amino acid sequence of GPCR6a was found to have 192 of 309 amino acid residues (62%) identical to, and 239 of 309 residues (77%) positive with, the 318 amino acid residue Odorant Receptor S46 from *Mus musculus* (SPTREMBL-ACC:Q9WU93) (E value =  $2.3e^{-103}$ ), and 152 of 302 amino acid residues (50%) identical to, and 211 of 302 residues (69%) positive with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (TREMBLNEW-ACC:AAD51279) (E value =  $1.8e^{-80}$ ).

GPCR6a also had homology to the proteins shown in the BLAST alignments in Table 6C.

Table 6C. BLASTX results for GPCR6a

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
patp:Y92365 G protein-coupled receptor protein 5 - Hom..+1		731	1.6e-71	1
patp:W01730 Human G-protein receptor HPRAJ70 - Homo sa..+1		730	2.0e-71	1
patp:W56641 G-protein coupled prostate tissue receptor..+1		730	2.0e-71	1
patp:R27875 Odorant receptor clone I14 - Rattus rattus..+1		495	1.6e-46	1
patp:R27868 Odorant receptor clone F5 - Rattus rattus,. +1		494	2.1e-46	1

**GPCR6b**

In the present invention, the target sequence identified previously, Accession Number  
5 AC020597D, was subjected to the exon linking process to confirm the sequence. PCR primers  
were designed by starting at the most upstream sequence available, for the forward primer, and  
at the most downstream sequence available for the reverse primer. In each case, the sequence  
was examined, walking inward from the respective termini toward the coding sequence, until a  
suitable sequence that is either unique or highly selective was encountered, or, in the case of  
10 the reverse primer, until the stop codon was reached. Such suitable sequences were then  
employed as the forward and reverse primers in a PCR amplification based on a wide range of  
cDNA libraries. The resulting amplicon was gel purified, cloned and sequenced to high  
redundancy to provide the sequence reported below, which is designated Accession Number  
AC020597D1. The resulting nucleotide sequence differs in 5 of 1012 bases given by  
15 Accession Number AC020597D, which leads to a difference in the amino acid sequence at  
residues 234 and 264.

The disclosed novel GPCR6b nucleic acid of 1012 nucleotides (also referred to as  
AC020597\_D1) is shown in Table 6D. An open reading begins with an ATG initiation codon  
at nucleotides 16-18 and ends with a TGA codon at nucleotides 991-993. A putative  
20 untranslated region upstream from the initiation codon and downstream from the termination  
codon are underlined in Table 6D, and the start and stop codons are in bold letters.

**Table 6D. GPCR6b Nucleotide Sequence (SEQ ID NO:23)**

```
GCATTACAAGCAGGATGTTCTTCCCAATGACACCCAGTTTCACCCCTCCTCCTTCTGTTGCTGGGGATCCCA
GGACTAGAAACACTTCACATCTGGATCGGCTTTCCTTCTGTGCTGTGTACATGATCGCACTCATAGGGAACCTC
ACTATTCTACTTGTGATCAAGACTGACAGCAGCCTACACCAGCCCATGTTCTACTTCTGGCCATGTTGGCCACC
ACTGATGTGGGTCTCTCAACAGCTACCATCCCTAAGATGCTTGGAACTCTTCTGGATCAACCTCAGAGGATCATC
TTTGAAGCCTGCCTACCCAGATGTTTTTTATCCAACTTCACACTTATGGAGTCAGCAGTCTTGTGGCAATG
GCTTATGACAGCTATGTGGCCATCTGCAATCCACTCCAATATAGCGCCATCCTCACCACAAAGTTGTTTCTGTG
ATTGGTCTTGGTGTGTTTGTGAGGGCTTAAATTTTCGTATCCCTCTATACTTCTTATATTGGGGTTGCCCTTC
TGTGGGAATCATGTAATCCCAACCTACTGTGAGCACATGGGTCTTGCTCATCTATCTTGTGCCAGCATCAAA
ATCAATATTATTTATGGTTTATGTCCATTTGTAATCTAGTGTGTTGACATCACAGTCATTGCCCTCTCTTATGTG
CATATTCTTGTGCTGTTTCCGTCTTCTACTCATGAAGCCCGACTCAAGTCCCTCAGCACATGTGGTTTCACAT
GTGTGTGTAATCCTTGCCTTCTATACACCAGCCCTCTTTCTTTATGACTCATCGCTTGGCCGAAATGTGCC
CGCTATATCCATATACTCCTAGCCAATCTCTATGTTGTGGTGCCACCAATGCTCAATCCTGTATATATGGAGTC
AGAACCAAGCAGATCTATAAATGTGTGAAGAAAATATTATTGCAGGAACAAGGAATGAAAAGGAAGAGTACCTA
ATACATACGAGGTTCTGAATGCAATTTTATGAAATTT
```

The GPCR6b protein encoded by SEQ ID NO:21 has 325 amino acid residues, and is presented using the one-letter code in Table 6E (SEQ ID NO:24). The Psort profile for GPCR6b predicts that this sequence has a signal peptide and is likely to be localized at the endoplasmic reticulum with a certainty of 0.6850 or plasma membrane with a certainty of 0.6400.. It seems to have an uncleavable N-terminal signal sequence. The most likely cleavage site for a peptide, if there was one, is between amino acids 55 and 56, TDS-SL based on the SignalP result. The molecular weight of GPCR6b is 36629.6 Daltons.

**Table 6E. Encoded GPCR6b protein sequence (SEQ ID NO:24).**

```
MELPNDTQFHPSSFLLGLPIGLETLHIWIGFPFCVYMIALIGNFTILLVIKTDSSLHQPMFYFLAMLATTDVGL
STATIPKMLGIFWINLRGIIFEACLTQMFHINFTLMESAVLVAMAYDSYVAICNPLQYSAILTNKVVSVIGLV
FVRALIEVIPSILLILRLPFCGNHVIPHTYCEHMGHLAHLSCASIKINIYGLCAICNLVFDITVIALSYVHILCA
VRLPPTHEARKLSLSTCGSHVCVILAFYTPALFSFMTHFRGNVPRYIHILLANLYVVVPEMLNPNVIYGVRTKQI
YKCVKILLQEQGMEKEEYLHTRF
```

BLASTP results include those listed in Table 6F.

**Table 6F BLASTP results for GPCR6b**

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
SPTREMBL-ACC:Q9WU93	ODORANT RECEPTOR S46 - Mus musculus	318	194/309 (62%)	241/309 (77%)	1.6e- 105
SPTREMBL-ACC:Q9WVD9	MOR 3'BETA1 - Mus musculus (Mouse)	326	172/298 (57%)	226/298 (75%)	4.4e- 94
SPTREMBL-ACC:Q9Y5P1	HOR 5'BETA3 - Homo sapiens (Human)	312	131/305 (42%)	195/305 (63%)	4.0e- 68

The disclosed GPCR6 protein has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 6G. The GPCR6 protein has significant identity to olfactory receptor (OR) proteins:



Table 6G. BLAST results for GPCR6					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 9935442 ref NP_064688.1	odorant receptor S46 gene [Mus musculus]	318	192/310 (61%)	239/310 (76%)	6e-98
gi 7305349 ref NP_038647.1	O olfactory receptor 67 [Mus musculus]	326	70/298 (57%)	224/298 (75%)	1e-86
gi 9938014 ref NP_064686.1	odorant receptor S18 gene [Mus musculus]	321	157/305 (51%)	225/305 (73%)	1e-83
gi 6532001 gb AAD27596.2 AF121976_1 (AF121976)	odorant receptor S19 [Mus musculus]	339	158/280 (56%)	209/280 (74%)	4e-83
gi 11908211 gb AAG41676.1  (AF137396)	HOR 5'Beta14 [Homo sapiens]	313	160/300 (53%)	221/300 (73%)	1e-82

This information is presented graphically in the multiple sequence alignment given in Table 6H (with GPCR6a being shown on line 1 and GPCR6b being shown on line 2) as a ClustalW analysis comparing GPCR6 with related protein sequences.

5

Table 6H Information for the ClustalW proteins:

- 1) GPCR6a (SEQ ID NO:22)
- 2) GPCR6b (SEQ ID NO:24)
- 3) gi|9935442|ref|NP\_064688.1| odorant receptor S46 gene [Mus musculus] (SEQ ID NO:53)
- 4) gi|7305349|ref|NP\_038647.1| olfactory receptor 67 [Mus musculus] (SEQ ID NO:57)
- 5) gi|9938014|ref|NP\_064686.1| odorant receptor S18 gene [Mus musculus] (SEQ ID NO:56)
- 6) gi|6532001|gb|AAD27596.2|AF121976\_1 (AF121976) odorant receptor S19 [Mus musculus] (SEQ ID NO:54)
- 7) gi|11908211|gb|AAG41676.1| (AF137396) HOR 5'Beta14 [Homo sapiens] (SEQ ID NO:55)

15

10	20	30	40	50	60
GPCR6a	.....	.....	.....	.....	.....
GPCR6b	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....
70	80	90	100	110	120
GPCR6a	.....	.....	.....	.....	.....
GPCR6b	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....
130	140	150	160	170	180
GPCR6a	.....	.....	.....	.....	.....
GPCR6b	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....

	190	200	210	220	230	240
GPCR6a	.....	.....	.....	.....	.....	.....
GPCR6b	.....	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
	250	260	270	280	290	300
GPCR6a	.....	.....	.....	.....	.....	.....
GPCR6b	.....	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
	310	320	330	340	350	360
GPCR6a	.....	.....	.....	.....	.....	.....
GPCR6b	.....	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
GPCR6a	.....	.....	.....	.....	.....	.....
GPCR6b	.....	.....	.....	.....	.....	.....
gi 9935442	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....

The presence of identifiable domains in GPCR6 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website

5 (http://www.ebi.ac.uk/interpro/).

DOMAIN results for GPCR6 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 6I with the statistics and domain description. The results indicate that GPCR6 has homology to the 7tm\_1 (InterPro) 7

10 transmembrane receptor (rhodopsin family) (as defined by Interpro) This indicates that the sequence of GPCR6 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

**Table 6I. DOMAIN results for GPCR6 against the consensus 7tm 1 domain (SEQ ID NO:76)**

CD-Length = 254 residues, 100.0% aligned  
Score = 77.4 bits (182), Expect = 1e-15

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10  
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30  
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(particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary

5 Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders of the like.

10 The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the OR -like protein may be useful in gene therapy, and the OR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have

15 efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright

20 Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

25 The novel nucleic acid encoding OR-like protein, and the OR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the

30 presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCRX Antibodies" section below. For

example the disclosed GPCR6 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR6 epitope is from about amino acids 170 to 180. In another embodiment, a GPCR6 epitope is from about amino acids 230 to 240. In additional embodiments, GPCR6 epitopes are from amino acids 250 to 280 and 290-310. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

## GPCR7

### GPCR7a

A novel GPCR nucleic acid was identified by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs, and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR7 nucleic acid of 968 nucleotides (also referred to as AC025249\_A) is shown in Table 7A. An open reading frame begins with an ATG initiation codon at nucleotides 6-8 and ends with a TAG codon at nucleotides 960-962. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 7A, and the start and stop codons are in bold letters.

**Table 7A. GPCR7a Nucleotide Sequence (SEQ ID NO:25)**

TCTTCATGATGGTGGATCCCAATGGCAATGAATCCAGTGCTACATACTTCATCCTAATAGGCCTCCCTGGTTTAGAAG  
 AGGCTCAGTTCTGGTTGGCCTTCCCATGTGCTCCCTCTACCTTATGCTGTGCTAGGFAACTTGACAATCATCTACA  
 TTGTGCGGACTGAGCACAGCCTGCATGAGCCCATGTATATATTTCTTTGCATGCTTTCAGGCATTGACATCCTCATCT  
 CCACCTCATCCATGCCAAAATGCTGGCCATCTTCTGGTTCAATTCCACTACCATCCAGTTTGATGCTTGTCTGCTAC  
 AGATGTTTGCCATCCACTCCTTATCTGGCATGGAATCCACAGTGCTGCTGGCCATGGCTTTTGACCGCTATGTGGCCA  
 TCTGTCACCCACTGCGCCATGCCACAGTACTTACGTTGCCTCGTGTCAACAAAATGGTGTGGCTGCTGTGGTGGCGGG  
 GGGCTGCACTGATGGACCCCTTCTGTCTTCATCAAGCAGCTGCCCTTCTGCCGCTCCAATATCCTTTCCCATTCCT  
 ACTGCCTACACCAAGATGTCATGAAGCTGGCCTGTGATGATATCCGGGTCAATGTCGTCTATGGCCTTATCGTCATCA  
 TCTCCGCCATTGGCCTGGACTCACTTCTCATCTCCTTCTCATATCTGCTTATTCTTAAGACTGTGTTGGGCTTGACAC  
 GTGAAGCCCAGGCCAAGGCATTGGCACTTGCCTCTCATGTGTGTGCTGTGTTTCATATTCTATGTACCTTTCATTG  
 GATTGTCCATGGTGCATCGCTTTAGCAAGCGGCGTGACTCTCCGCTGCCGTCATCTTGCCCAATATCTATCTGCTGG  
 TTCCTCCTGTGCTCAACCAATTGTCTATGGAGTGAAGACAAAGGAGATTCGACAGCGCATCCTTCGACTTTTCCATG  
 TGGCCACACACGCTTCAGAGCCCTAGGTGTCA

GPCR7a has high homology to several other nucleic acids including those in the BLASTN alignments described in Table 7B.

Table 7B. BLASTN results for GPCR7a				
Gene Index/ Identifier	Protein/ Organism	Length (bp)	Identity (%)	Expect
gb:GENBANK- ID:AF079864 acc:AF0 79864	Rattus norvegicus putative G- protein coupled receptor RAlc mRNA, complete cds	2910	596/879 (67%)	2.4e-68
gb:GENBANK- ID:AR009514 acc:ARO 09514	Sequence 1 from patent US 5756309	1474	594/874 (67%)	7.0e-69
patn:X40518	Human secreted protein 5' EST	381	242/242 (100%)	3.8e-49
patn:X40528	Human secreted protein 5' EST	399	280/286 (97%)	4.1e-57
gb:GENBANK- ID:AI557139 acc:AI5 57139	PT2.1_13_H11.r tumor2 Homo sapiens cDNA 3', mRNA sequence - Homo sapiens	1000	165/173 (95%)	5.5e-28

The GPCR7a protein encoded by SEQ ID NO:25 has 318 amino acid residues, and is presented using the one-letter code in Table 7B (SEQ ID NO:26). The SignalP, Psort and/or Hydropathy profile for GPCR7a predict that GPCR7a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence is coded for in the first 44 amino acids, *i.e.*, with a cleavage site at VRT-EH, between amino acids 54 and 55. This is typical of this type of membrane protein. The molecular weight of GPCR7b is 35401.9 Daltons.

Table 7C. Encoded GPCR7 protein sequence (SEQ ID NO:26).
MMVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGDIL ISTSSMPKMLAIFWENSTTIQFDACLLQMFHSLSGMESTVLLAMAFDRYVAICHPLRHATVLTLPRTKIGVA AVVRGAALMAPLPVFQKLPFCRSNLSHSYCLHQDVMKLCDDIRVNVVYGLIVIIISAIGLDSLLISFSYLLIL KTVLGLTREAQAKAFGTCVSHVCAVFIFVVPFGLSMVHRFSKRDSPLPVILANIYLLVPPVLPNPIVYGVKTK IRQRILRLFHVATHASEP

The full amino acid sequence of the protein of the invention was found to have high homology to a number of polypeptides including the ones in the BLASTX alignments in Table 7D and 7E.

Table 7D. BLAST results for GPCR7a					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ptnr:SPTREMBL- ACC:088628	PUTATIVE G- PROTEIN COUPLED RECEPTOR RA1C - Rattus norvegicus (Rat)	320	183/305 (60%)	235/305 (77%)	1.5e- 98
patp:W01730	Human G-protein receptor HPRAJ70 - Homo sapiens	320	181/305 (59%)	235/305 (77%)	1.8e- 97
patp:Y11796	Human 5' EST secreted protein	21	20/20 (100%)	20/20 (100%)	1.3e- 05
gi 6532001 gb AAD27 596.2 AF121976_1 (AF121976)	Odorant receptor S19 [Mus musculus]	339	158/280 (56%)	209/280 (74%)	4e-83

Table 7E. BLASTX results for GPCR7a				
Sequences producing High-scoring Segment Pairs:		Reading Frame	High Score	Smallest Sum Prob P(N)      N
Patp:Y92365 G protein-coupled receptor protein 5 - Hom		+3	1623	4.8e-166    1
Patp:W01730 Human G-protein receptor HPRAJ70 - Homo sa.		+3	971	5.9e-97    1
Patp:W56641 G-protein coupled prostate tissue receptor.		+3	971	5.9e-97    1
patp:R27875 Odorant receptor clone I14 - Rattus rattus.		+3	425	4.2e-39    1

### GPCR7b and c

- 5 In the present invention, the target sequence identified previously, Accession Number AC025249\_A, was subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding
- 10 sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in PCR amplifications based on a library containing a wide range of cDNA species. The resulting two amplicons were gel purified, cloned and sequenced to high redundancy to provide the sequences reported
- 15 below, which are designated GPCR7b (Accession Numbers AC025249\_A1) and GPCR7c (AC025249\_A\_da3). The sequence of GPCR7b contains one amino acid difference from that of GPCR7a at position 247, wherein alanine is replaced by valine. The sequence of GPCR7c has one amino acid different at position 183, wherein leucine is replaced by proline.

**GPCR7b**

A novel GPCR nucleic acid was identified by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs, and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR7 nucleic acid of 969 nucleotides (also referred to as AC025249\_A1) is shown in Table 7F. An open reading frame begins with an ATG initiation codon at nucleotides 7-9 and ends with a TAG codon at nucleotides 961-963. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 7F, and the start and stop codons are in bold letters.

**Table 7F. GPCR7 Nucleotide Sequence (SEQ ID NO:27)**

TTCTTCATGATGGTGGATCCCAATGGCAATGAATCCAGTGCTACATACTTCATCCTAATAGGCCTCCCTGGTTAGAA  
 GAGGCTCAGTTCTGGTTGGCCTTCCCATTTGTGCTCCCTCTACCTTATTGCTGTGCTAGGTAAGTGAACATCATCTAC  
 ATTTGTGCGACTGAGCACAGCCTGCAATGAGCCCATGTATATATTTCTTTGCATGCTTTCAGGCATTGACATCCTCATC  
 TCCACCTCATCCATGCCCAAAATGCTGGCCATCTTCTGGTTCAATTCCACTACCATCCAGTTTGATGCTTGTCTGCTA  
 CAGATGTTTGGCCATCCACTCCTTATCTGGCATGGAATCCACAGTGCTGCTGGCCATGGCTTTTGACCGCTATGTGGCC  
 ATCTGTCAACCACTGCGCCATGCCACAGTACTTACGTTGCCCTCGTGTCAACCAAAATTGGTGTGGCTGCTGTGGTGGCG  
 GGGCTGCACTGATGGCACCCCTTCTGTCTTCATCAAGCAGCTGCCCTTCTGCCGCTCCAATATCCTTCCCATTC  
 TACTGCCACACCAAGATGTATGAAGCTGGCCTGTGATGATATCGGGTCAATGTCGTCTATGGCCTTATCGTCATC  
 ATCTCCGCCATTGGCCTGGACTCACTTCTCATCTCCTTCTCATATCTGCTTATCTTAAGACTGTGTGGGCTTGACA  
 CGTGAAGCCCAAGGCAAGGCATTGGCACTTGGCTCTCTCATGTGTGCTGTGTTTCATATTCTATGTACCTTTTCATT  
 GGATTGTCCATGGTGCATCGCTTTAGCAAGCGCGTGAAGTCTCCACTGCCCGTCATCTTGGCCAATATCTATCTGCTG  
 GTTCTCCTGTGCTCAACCAATTGTCTATGGAGTGAAGACAAAGGAGATTCGACAGCGCATCCTTCGACTTTTCCAT  
 GTGGCCACACAGCTTCAGAGCCCTAGGTGTCA

The GPCR7b protein encoded by SEQ ID NO:27 has 318 amino acid residues, and is presented using the one-letter code in Table 7G (SEQ ID NO:28). The SignalP, Psort and/or Hydropathy profile for GPCR7a predict that GPCR7a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence is coded for in the first 44 amino acids, *i.e.*, with a cleavage site at VRT-EH, between amino acids 54 and 55. This is typical of this type of membrane protein. The molecular weight of GPCR7b is 35385.8 Daltons.

**Table 7G. Encoded GPCR7b protein sequence (SEQ ID NO:28).**

MMVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDIL  
 ISTSSMPKMLAIFWFNSTTIQFDACLLQMFATHSLSGMESTVLLAMAFDRYVAICHPLRHATVLTLPVTKIGVA  
 AVVRGAALMAPLPVFIKQLFFCRSNILSHSYCPHQDVMKCLACDDIRVNVVYGLIVIIISAIGLDSLLISFSYLLIL  
 KTVLGLTREAQAKAFGTCVSHVCAVFIFVYFFIGLSMVHRESKRRDSPLPVILANIYLLVPPVLNPIVYGVKTE  
 IRQRLRLHFVATHASEP



The full amino acid sequence of GPCR7b was found to have 152 of 301 amino acid residues (50%) identical to, and 213 of 301 residues (70%) positive with, the 319 amino acid residue Putative G-Protein Coupled Receptor RA1C from *Rattus norvegicus* (SPTREMBL-ACC:O88628) (E value =  $3.3e^{-81}$ ), and 142 of 295 amino acid residues (48%) identical to, and 199 of 295 residues (67%) positive with, the 312 amino acid residue Olfactory Receptor HPFH1OR from *Homo sapiens* (sptrEMBL-Q9UKL2) (E value =  $2.9e^{-75}$ )

GPCR7b also has high homology to the following proteins found through BLASTX alignments, shown in Table 7H.

Table 7H. BLASTX results for GPCR7b				
Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
Ptnr: SPTREMBL-ACC:O88628 PUTATIVE G-PROTEIN COUPLED RE.	+1	981	6.6e-98	1
ptnr: SPTREMBL-ACC:Q9YH55 OLFACTORY RECEPTOR-LIKE PROTE.	+1	816	2.0e-80	1
ptnr: SPTREMBL-ACC:Q9WVD9 MOR 3'BETA1 - Mus musculus (M.	+1	812	5.3e-80	1
ptnr: SPTREMBL-ACC:Q9WU90 ODORANT RECEPTOR S19 - Mus mu.	+1	799	1.3e-78	1
ptnr: SPTREMBL-ACC:Q9WVN5 MOR 5'BETA2 - Mus musculus (M.	+1	777	2.7e-76	1
ptnr: SPTREMBL-ACC:Q9WVD8 MOR 3'BETA2 - Mus musculus (M	+1	769	1.9e-75	1
ptnr: SPTREMBL-ACC:Q9WU89 ODORANT RECEPTOR S18 - Mus mu.	+1	769	1.9e-75	1
ptnr: SPTREMBL-ACC:Q9WVD7 MOR 3'BETA3 - Mus musculus (M.	+1	764	6.5e-75	1
ptnr: SPTREMBL-ACC:Q9UKL2 OLFACTORY RECEPTOR HPFH1OR -	+1	760	1.7e-74	1
ptnr: SPTREMBL-ACC:Q9WVN6 MOR 5'BETA3 - Mus musculus (M.	+1	756	4.6e-74	1
ptnr: SPTREMBL-ACC:Q9Y5P1 HOR 5'BETA3 - Homo sapiens (H.	+1	723	1.4e-70	1
ptnr: SPTREMBL-ACC:Q9WVN4 MOR 5'BETA1 - Mus musculus (M.	+1	707	7.1e-69	1
ptnr: SPTREMBL-ACC:Q9WU93 ODORANT RECEPTOR S46 - Mus mu.	+1	701	3.1e-68	1
ptnr: SPTREMBL-ACC:Q9Y5P0 HOR 5'BETA1 - Homo sapiens (H.	+1	630	1.0e-60	1
ptnr: SPTREMBL-ACC:Q9WU94 ODORANT RECEPTOR S50 - Mus mu.	+1	578	3.3e-55	1

## 10 GPCR7c

The disclosed novel GPCR7c nucleic acid of 968 nucleotides (also referred to as AC025249\_Ada3) is shown in Table 7I. An open reading frame begins with an ATG initiation codon at nucleotides 7-9 and ends with a TAG codon at nucleotides 961-963. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 7I, and the start and stop codons are in bold letters.

Table 7I. GPCR7 Nucleotide Sequence (SEQ ID NO:29)
<p>TTCTTCATGATGGTGGATCCCAATGGCAATGAATCCAGTGCTACATACTTCATCCTAATAGGCCTCCCTGGTTAGAA  GAGGCTCAGTTCTGGTTGGCCTTCCCATTTGCTCCCTCTACCTTATTGCTGTGCTAGGTAAGTTGACAATCATCTAC  ATTGTGCGGACTGAGCACAGCCTGCATGAGCCCATGTATATATTCTTTGCATGCTTTCAGGCATTGACATCCTCATC  TCCACCTCATCCATGCCCAAATGCTGGCCATCTTCTGGTTCAATCCACTACCATCCAGTTTGATGCTGTCTGCTA  CAGATGTTTGCCATCCACTCCTTATCTGGCATGGAATCCACAGTGCTGCTGGCCATGGCTTTTGACCGCTATGTGGCC  ATCTGTCAACCACTGCGCCATGCCACAGTACTTACGTGGCTCGTGTACCAAATTTGGTGTGGCTGCTGGTGGCGG  GGGGCTGCATGTATGGCACCCCTTCTGTCTTCATCAAGCAGCTGCCCTTCTGCCGCTCCAATATCCTTTCCCATTC  TACTGCCCCACCAAGATGTCATGAAGTGGCTGTGATGATATCGGGTCAATGTCGTCTATGGCCTTATCGTCATC  ATCTCGCCATTGGCCTGGACTCACTTCTCATCTCCTTCTCATATCTGCTTATCTTAAGACTGTGTTGGGCTTGACA  CGTGAAGCCAGGCCAAGGCATTGGCACTTGCGTCTCTCATGTGTGTGCTGTGTTCATATTCTATGTACCTTTTCAAT  GGATTGTCCATGTGTCATCGCTTAGCAAGCGCGTGACTCTCCACTGCCGTCATCTTGGCCCAATATCTATCTGCTG  GTTCTCTGTGCTCAACCAATTTGCTATGGAGTGAAGACAAAGGAGATTCGACAGCGCATCCTTCGACTTTTCCAT  GTGGCCACACACGCTTCAGAGCCCTAGGTGTA</p>

The GPCR7c protein encoded by SEQ ID NO:29 has 318 amino acid residues, and is presented using the one-letter code in Table 7J (SEQ ID NO:30). The SignalP, Psort and/or Hydropathy profile for GPCR7a predict that GPCR7a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence is coded for in the first 44 amino acids, *i.e.*, with a cleavage site at VRT-EH, between amino acids 54 and 55. This is typical of this type of membrane protein. The molecular weight of GPCR7c is 35385.8 Daltons.

**Table 7J. Encoded GPCR7c protein sequence (SEQ ID NO:30).**

```
MMVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDIL
ISTSSMPKMLAIFWENSTTIQFDACLLQMFALHSLSGMESTVLLAMAFDRYVAICHPLRHATVLTLPRTKIGVA
AVVRGAALMAPLPVFIEKQLPFCRSNILSHSYCPHQDVMKLACDDIRVNVVYGLVIVISAIGLDSLLISFSYLLIL
KTVLGLTREAQAKAFGTCVSHVCAVFIFYVPFGLSMVHRFSKRSDPLFVILANIYLLVPPVLNPIVGVKTKKE
IRQRILRLHFVATHASEP
```

Homologous proteins to GPCR7c were searched for using BLASTX. Some of the alignments are included in Table 7K.

**Table 7K. BLAST results for GPCR7c**

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ptnr:SPTREMBL- ACC:O88628	PUTATIVE G- PROTEIN COUPLED RECEPTOR RA1C - Rattus norvegicus (Rat)	320	183/305 (60%)	235/305 (77%)	1.5e- 98
ptnr:SPTREMBL- ACC:Q9YH55	OLFACTORY RECEPTOR-LIKE PROTEIN COR3'BETA - Gallus gallus s	319	152/301 (50%)	213/301 (70%)	3.3e- 81
ptnr:SPTREMBL- ACC:Q9UKL2	OLFACTORY RECEPTOR HPFH1OR - Homo sapiens	312	142/295 (48%)	199/295 (67%)	2.9e- 75

GPCR7c also has high homology to the following proteins found through BLASTX alignments, shown in Table 7L.

**Table 7L. BLASTX results for GPCR7b**

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
ptnr:SPTREMBL-ACC:O88628 PUTATIVE G-PROTEIN COUPLED RE.+1		981	6.8e-98	1
ptnr:SPTREMBL-ACC:Q9YH55 OLFACTORY RECEPTOR-LIKE PROTE.+1		816	2.1e-80	1
ptnr:SPTREMBL-ACC:Q9WVD9 MOR 3'BETA1 - Mus musculus (M.+1		812	5.5e-80	1
ptnr:SPTREMBL-ACC:Q9WU90 ODORANT RECEPTOR S19 - Mus mu.+1		799	1.3e-78	1

Possible SNPs found for GPCR7c are listed in Table 7M.

Table 7M: SNPs			
Consensus Position	Depth	Base Change	PAF
446	18	A>-	0.111
513	17	T>C	0.118
555	16	T>C	0.125
827	9	A>G	0.333

The disclosed GPCR7 protein has good identity with a number of olfactory receptor proteins. The identity information used for ClustalW analysis is presented in Table 7O. The GPCR7 protein has significant identity to olfactory receptor (OR) proteins (Table 7N):

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Table 7N. BLAST results for GPCR7					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 3420759 gb AAD12761.1  (AF079864)	putative G-protein coupled receptor RA1c [Rattus norvegicus]	320	173/299 (57%)	221/299 (73%)	3e-88
gi 11875778 gb AAG40776.1 AF311306_1 (AF311306)	prostate specific G-protein coupled receptor; PSGR [Homo sapiens]	320	173/299 (57%)	223/299 (73%)	5e-88
gi 11908213 gb AAG41678.1  (AF137396)	HOR5'Beta12 [Homo sapiens]	312	157/304 (51%)	205/304 (66%)	4e-76
gi 11908220 gb AAG41684.1  (AF133300)	MOR 3'Beta4 [Mus musculus]	319	156/308 (50%)	207/308 (66%)	6e-73
gi 11908214 gb AAG41679.1  (AF137396)	HOR5'Beta11 [Homo sapiens]	314	143/310 (46%)	207/310 (66%)	2e-69

This information is presented graphically in the multiple sequence alignment given in Table 7O (with GPCR7 being shown on line 1) as a ClustalW analysis comparing GPCR7 with related protein sequences.

10

**Table 7O. Information for the ClustalW proteins:**

- 1) GPCR7a (SEQ ID NO:26)
- 2) GPCR7b (SEQ ID NO:28)
- 2) gi|3420759|gb|AAD12761.1| (AF079864) putative G-protein coupled receptor RA1c [Rattus norvegicus] (SEQ ID NO:64)
- 3) gi|11875778|gb|AAG40776.1|AF311306\_1 (AF311306) prostate specific G-protein coupled receptor; PSGR [Homo sapiens] (SEQ ID NO:65)
- 4) gi|11908213|gb|AAG41678.1| (AF137396) HOR5'Beta12 [Homo sapiens] (SEQ ID NO:61)
- 5) gi|11908220|gb|AAG41684.1| (AF133300) MOR 3'Beta4 [Mus musculus] (SEQ ID NO:58)
- 6) gi|11908214|gb|AAG41679.1| (AF137396) HOR5'Beta11 [Homo sapiens] (SEQ ID NO:60)

20

	10	20	30	40	50	60
GPCR7a	MMVDPKNGESSATYFLLIGLPGLESAQFLLAFPLCSLYLTAVLGNLTHYIVRTERSLHE					
GPCR7b	MMVDPKNGESSATYFLLIGLPGLESAQFLLAFPLCSLYLTAVLGNLTHYIVRTERSLHE					
gi 3420759	----MSSCNFTHATFVLLIGIPGLESAHFEGFPLLSKYVAVAEGNCIYFIVRTERSLHA					
gi 11875778	----MSSCNFTHATFVLLIGIPGLESAHFEGFPLLSKYVAVAEGNCIYFIVRTERSLHA					
gi 11908213	---MGLFNVTHTPFFLLIGIPGLESHSASGGLCVVAVAAGGNIVLQAVRVGPSLHE					
gi 11908220	-MATNSSTIVSTFVLLIGIPGVDFHHRHISIEFCCLVVGHTGNCMIGHIVRTPRLHE					
gi 11908214	-MLGLNGTPFPQPTLQLTGIPGIOTGLTVALIFCCLVVAISVGNLSHTLVTFEPFLHC					
	70	80	90	100	110	120
GPCR7a	PMYIFLCMLAGIDHISTSSMPKMLATFWNSITTCFACLRQMFATHSLSGMESVLLA					
GPCR7b	PMYIFLCMLAGIDHISTSSMPKMLATFWNSITTCFACLRQMFATHSLSGMESVLLA					
gi 3420759	PMYIFLCMLAIDCHASTSTMPKLLALFWDSREITFDACLRQMFATHSLSAESVLLA					
gi 11875778	PMYIFLCMLAIDCHASTSTMPKLLALFWDSREITFDACLRQMFATHSLSAESVLLA					
gi 11908213	PMYIFLCMLAIDCHASTSTMPKLLALFWDSREITFDACLRQMFATHSLSAESVLLA					
gi 11908220	PMYIFLCMLAIDCHASTSTMPKLLALFWDSREITFDACLRQMFATHSLSAESVLLA					
gi 11908214	PMYIFLCMLAIDCHASTSTMPKLLALFWDSREITFDACLRQMFATHSLSAESVLLA					
	130	140	150	160	170	180
GPCR7a	MAFDRYVAICHPLRHATVLTLPRTKIGVAAYVFGHALAPLPVFIKQLPFCRSNVLSHS					
GPCR7b	MAFDRYVAICHPLRHATVLTLPRTKIGVAAYVFGHALAPLPVFIKQLPFCRSNVLSHS					
gi 3420759	MAFDRYVAICHPLRHATVLTLPRTKIGVAAYVFGHALAPLPVFIKQLPFCRSNVLSHS					
gi 11875778	MAFDRYVAICHPLRHATVLTLPRTKIGVAAYVFGHALAPLPVFIKQLPFCRSNVLSHS					
gi 11908213	MAFDRYVAICHPLRHATVLTLPRTKIGVAAYVFGHALAPLPVFIKQLPFCRSNVLSHS					
gi 11908220	MAFDRYVAICHPLRHATVLTLPRTKIGVAAYVFGHALAPLPVFIKQLPFCRSNVLSHS					
gi 11908214	MAFDRYVAICHPLRHATVLTLPRTKIGVAAYVFGHALAPLPVFIKQLPFCRSNVLSHS					
	190	200	210	220	230	240
GPCR7a	YCLHODVYKLCADDIRVNVYGLIVTISAIGLSTLISFSYLLIKTVLGIT-REACAKA					
GPCR7b	YCLHODVYKLCADDIRVNVYGLIVTISAIGLSTLISFSYLLIKTVLGIT-REACAKA					
gi 3420759	YCLHODVYKLCADDIRVNVYGLIVTISAIGLSTLISFSYLLIKTVLGIT-REACAKA					
gi 11875778	YCLHODVYKLCADDIRVNVYGLIVTISAIGLSTLISFSYLLIKTVLGIT-REACAKA					
gi 11908213	YCLHODVYKLCADDIRVNVYGLIVTISAIGLSTLISFSYLLIKTVLGIT-REACAKA					
gi 11908220	YCLHODVYKLCADDIRVNVYGLIVTISAIGLSTLISFSYLLIKTVLGIT-REACAKA					
gi 11908214	YCLHODVYKLCADDIRVNVYGLIVTISAIGLSTLISFSYLLIKTVLGIT-REACAKA					
	250	260	270	280	290	300
GPCR7a	FGTCVSHYCAVFIYFVEFGLSVVHRESRRDSPFVLENTYLLVPPVLNPIYGVKTK					
GPCR7b	FGTCVSHYCAVFIYFVEFGLSVVHRESRRDSPFVLENTYLLVPPVLNPIYGVKTK					
gi 3420759	FGTCVSHYCAVFIYFVEFGLSVVHRESRRDSPFVLENTYLLVPPVLNPIYGVKTK					
gi 11875778	FGTCVSHYCAVFIYFVEFGLSVVHRESRRDSPFVLENTYLLVPPVLNPIYGVKTK					
gi 11908213	FGTCVSHYCAVFIYFVEFGLSVVHRESRRDSPFVLENTYLLVPPVLNPIYGVKTK					
gi 11908220	FGTCVSHYCAVFIYFVEFGLSVVHRESRRDSPFVLENTYLLVPPVLNPIYGVKTK					
gi 11908214	FGTCVSHYCAVFIYFVEFGLSVVHRESRRDSPFVLENTYLLVPPVLNPIYGVKTK					
	310	320				
GPCR7a	EIRQRILRLHVRTHASEP----					
GPCR7b	EIRQRILRLHVRTHASEP----					
gi 3420759	EIRQRILRLHVRTHASEP----					
gi 11875778	EIRQRILRLHVRTHASEP----					
gi 11908213	EIRQRILRLHVRTHASEP----					
gi 11908220	EIRQRILRLHVRTHASEP----					
gi 11908214	EIRQRILRLHVRTHASEP----					

The presence of identifiable domains in GPCR7 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR7 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 7P with the statistics and domain

description. The results indicate that this protein contains homology to the following protein domains (as defined by Interpro) at the indicated positions: domain name 7tm\_1 (InterPro) 7 transmembrane receptor (rhodopsin family). This indicates that the sequence of GPCR7 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

**Table 7P. DOMAIN results for GPCR7 against the consensus 7tm  
1 domain (SEQ ID NO:77)**

CD-Length = 254 residues, 100.0% aligned  
Score = 77.4 bits (189), Expect = 1e-15

		10	20	30	40	50	60
10	GPCR7 (SEQ ID NO:26)	G	N	L	T	I	F
	Consensus 7tm 1 domain	G	N	L	T	I	F
		70	80	90	100	110	120
15	GPCR7	L	O	M	F	I	H
	Consensus 7tm 1 domain	L	O	M	F	I	H
		130	140	150	160	170	180
20	GPCR7	P	L	F	I	K	O
	Consensus 7tm 1 domain	P	L	F	I	K	O
		190	200	210	220	230	240
25	GPCR7	I	S	F	S	V	L
	Consensus 7tm 1 domain	I	S	F	S	V	L
		250	260	270	280	290	300
30	GPCR7						
	Consensus 7tm 1 domain						
		310	320	330	340	350	360
35	GPCR7						
	Consensus 7tm 1 domain						
		370	380	390			
40	GPCR7	H	R	F	S	K	R
	Consensus 7tm 1 domain	H	R	F	S	K	R

The similarity information for the GPCR7 protein and nucleic acid disclosed herein suggest that GPCR7 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in

gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon. The novel nucleic acid encoding GPCR7, and the GPCR7 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

The nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in used in the treatment of infections such as bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The disclosed GPCR7 polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies

that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. For example the disclosed GPCR7 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR7 epitope is from about amino acids 50 to 60. In another embodiment, a GPCR7 epitope is from about amino acids 170 to 200. In additional embodiments, GPCR7 epitopes are from amino acids 260 to 280 and 290-310. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

## GPCR8

### GPCR8a

A novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR8a nucleic acid of 980 nucleotides (also referred to as AC025249\_B) is shown in Table 8C. An open reading frame begins with an ATG initiation codon at nucleotides 3-5 and ends with a TGA codon at nucleotides 975-977. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 8A, and the start and stop codons are in bold letters.

**Table 8A. GPCR8a Nucleotide Sequence (SEQ ID NO:31)**

```

TGATGCTGGGTCCAGCTTACAACCACACAATGGAAACCCCTGCCTCCTTCTCCTTGTGGGTATCCCAGGACTGCA
ATCTTCACATCTTTGGCTGGCTATCTCACTGAGTGCCATGTACATCACAGCCCTGTTAGGAAACACCCTCATCGTG
ACTGCAATCTGGATGGATTCCACTCGGCATGAGCCCATGTATTGCTTTCTGTGTGTTCTGGCTGCTGAGACATTG
TTATGGCCTCCTCCGTGGTACCAAGATGGTGAGCATCTTCTGCTCGGGAGACAGCTCCATCAGCTTTAGTGCTTG
TTTCACTCAGATGTTTTTGTCCACTTAGCCACAGCTGTGGAGACGGGGCTGCTGCTGACCATGGCTTTTGACCCG
TATGTAGCCATCTGCAAGCCTCTACACTACAAGAGAATTCTACGCCTCAAGTATGCTGGGAATGAGTATGGCCG
TCACCATCAGAGCTGTACATTGACTCCACTGAGTTGGATGATGAATCATCTACCTTTCTGTGGCTCCAATGT
GGTTGTCCACTCCTACTGTAAGCACATAGCTTTGGCCAGGTTAGCATGTGCTGACCCCGTCCCAGCAGTCTCTAC
AGTCTGATTGGTTCTCTCTTATGGTGGGCTCTGATGTGGCCTTCATTGCTGCCTCCTATATCTTAATTCTCAGGG
CAGTATTTGATCTCTCCTCAAAGACTGCTCAGTTGAAAGCATTAAAGCACATGTGGCTCCCATGTGGGGTTATGGC
TTTGTAATCTACTACCTGGGATGGCATCCATCTATGCGGCCTGGTTGGGGCAGGATATAGTGCCCTTGACACACCAA
GTGCTGCTAGCTGACCTGTACGTGATCATCCAGCCACTTTAAATCCCATCATCTATGGCATGAGGACCAACA
ATTGCTGGAGGGAATATGGAGTTATCTGATGCACTTCTCTTTGACCACTCCAACCTGGGTTCATGAACA

```

The disclosed nucleic acid sequence has 537 of 858 bases (62%) identical to a *Mus musculus* odorant receptor S19 gene, complete cds:(GENBANK-ID:AF121976|acc:AF121976) (E value =  $3.5e^{-41}$ ).

5 The GPCR8a protein encoded by SEQ ID NO:31 has 324 amino acid residues, and is presented using the one-letter code in Table 8B (SEQ ID NO:32). The SignalP, Psort and/or Hydropathy profile for GPCR8a predict that GPCR8a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence with a cleavage site at the slash in the sequence VTA-IW, between amino acids 52 and 53. This is typical of this type of membrane protein. The molecular weight of GPCR8a is 10 35385.6 Daltons.

**Table 8B. Encoded GPCR8a protein sequence (SEQ ID NO:32).**

```

MLGPAYNHTMETPASFLLVGIPGLQSSHLWLAI SLSAMYITALLGNTLIVTAIWMDSTRHEPMYCFLCVLAAVDIV
MASSVVPKMVSIFCSGDSSISFSACFTQMFFVHLATAVETGLLLTMAFDYVAICKPLHYKRILTPQVLMGSMVA
TIRAVTFMTPLSWMMNHLFP CGSNVVVHSYCKHIALARLACADVPSSLSLIGSSIMVGS DVA FIAASYILILRA
VFDLSSKTAQLKALSTCGSHVGMALYYLP GMASIYA AWLGQDIVLHTQVLLADLYVII PATLNPIIYGMRTKQL
LEGIWSYLMHFLFDHSNLGS

```

15 The full amino acid sequence of the protein of the invention was found to have 142 of 308 amino acid residues (46%) identical to, and 202 of 306 residues (65%) positive with, the 321 amino acid residue Odorant Receptor S18 from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU89) (E value =  $1.5e^{-71}$ ), and 123 of 293 amino acid residues (41%) identical to, and 192 of 293 residues (65%) positive with, the 321 amino acid residue G-protein coupled prostate tissue receptor designated HPRAJ70 from *Homo sapiens* (patp:W56641) (E value = 20  $1.9e^{-65}$ ).

GPCR8a also has homology to the following proteins shown in the BLAST alignments in Table 8C.



Table 8C. BLASTX results for GPCR8a

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
patp:W01730 Human G-protein receptor HPRAJ70 - Homo sa +2		676	1.1e-65	1
patp:W56641 G-protein coupled prostate tissue receptor.+2		676	1.1e-65	1
patp:Y92365 G protein-coupled receptor protein 5 - Hom.+2		648	9.9e-63	1
patp:Y90873 Human G protein-coupled receptor GTAR14-3 .+2		425	4.2e-39	1

**GPCR8b**

A novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probes or homologs and run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR8b nucleic acid of 985 nucleotides (also referred to as AC025249\_C) is shown in Table 8C. An open reading frame begins with an ATG initiation codon at nucleotides 5-7 and ends with a TGA codon at nucleotides 977-979. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 8D, and the start and stop codons are in bold letters.

Table 8D. GPCR8b Nucleotide Sequence (SEQ ID NO:33)

TGTGATGCTGGGTCCAGCTTATAACCAACACAATGGAACCCCTGCCTCCTTCTCCTTGTGGGTATCCCAGGACTG  
CAATCTTCACATCTTTGGCTGGCTATCTCACTGAGTGCCATGTACATCATAGCCCTGTTAGGAAACACCATCATCG  
TGACTGCAATCTGGATGGATTCCACTCGGCATGAGCCCATGTATTGCTTTCTGTGTGTTCTGGCTGCTGTGGACAT  
TGTTATGGCTCCTCGGTGGTACCCAAGATGGTGAGCATCTTCTGCTCAGGAGACAGCTCAATCAGCTTTAGTGCT  
TGTTTCACTCAGATGTTTTTGTCCACTTAGCCACAGCTGTGGAGACGGGGCTGCTGCTGACCATGGCTTTTGACC  
GCTATGTAGCCATCTGCAAGCCTCTACACTACAAGAGAATTCTCAGCCCTCAAGTGATGCTGGGAATGAGTATGGC  
CATCACCATCAGAGCTATCATAGCCATAACTCCACTGAGTTGGATGGTGAGTCACTACCTTTCTGTGGCTCCAAT  
GTGGTTGTCCACTCCTACTGTGAGCACATAGCTTTGGCCAGGTTAGCATGTGCTGACCCCGTGCCAGCAGTCTCT  
ACAGTCTGATTGGTTCTCTCTTATGGTGGGCTCTGATGTGGCCTTCATTGCTGCCTCCTATATCTTAATTCTCAA  
GGCAGTATTTGGTCTCTCCTCAAAGACTGCTCAGTTGAAAGCATTAAAGCACATGTGGCTCCCATGTGGGGGTTATG  
GCTTTGTACTATCTACCTGGGATGGCATCCATCTATGCGGCCTGGTTGGGGCAGGATGTAGTGCCCTTGACACCCC  
AAGTCCTGCTAGCTGACCTGTACGTGATCATCCAGCCACCTTAATCCCATCATCTATGGCATGAGGACCAACA  
ACTGCGGGAGAGAATATGGAGTTATCTGATGCATGCTCTTTGACCATCCAACCTGGGTTTCATGAACACAA

The disclosed nucleic acid sequence has 533 of 858 bases (62%) identical to a *Mus musculus* odorant receptor S19 gene, complete cds:(GENBANK-ID:AF121976|acc:AF121976) (E value = 3.5e<sup>-41</sup>).

The GPCR8b protein encoded by SEQ ID NO:33 has 324 amino acid residues, and is presented using the one-letter code in Table 8E (SEQ ID NO:34). The SignalP, Psort and/or Hydropathy profile for GPCR8b predict that GPCR8b has a signal peptide and is likely to be

localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence with a cleavage site at the slash in the sequence VTA-IW, between amino acids 52 and 53. This is typical of this type of membrane protein. The molecular weight of GPCR8b is 35279.5 Daltons.

5

**Table 8E. Encoded GPCR8b protein sequence (SEQ ID NO:34).**

MLGPAYNHTMETPASFLLVGIPGLQSSHLWLAISSAMYIIALLGNTIIIVTAIWMDSTRHEPMYCFLCVLAADVIV MASSVVPKMVSIFCSGDSSISFSACFTQMFFVHLATAVETGLLLTMAFDRYVAICKPLHYKRILTPQVLMGMSMAI TIRAIITPLSWMVSHLPFCGSNVVHSYCEHIALARLACADVPSSLYSLIGSSLMVGSDVAFIAASYILILKA VFGLSSKTAQLKALSTCGSHVGMALYYLPGMASIYAÄWLQDQVVLHTQVLLADLYVIIPATLNPIIYGMRTKQL RERIWSYLMHVLFDSNLGS
---

The full amino acid sequence of the protein of the invention was found to have 146 of 306 amino acid residues (47%) identical to, and 202 of 306 residues (66%) positive with, the 321 amino acid residue Odorant Receptor S18 from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU89) (E value =  $8.2e^{-73}$ ), and 124 of 297 amino acid residues (41%) identical to, and 196 of 297 residues (65%) positive with, the 320 amino acid residue G-protein coupled prostate tissue receptor designated HPRAJ70 from *Homo sapiens* (patp:W56641) (E value =  $3.4e^{-66}$ ).

#### GPCR8c

In the present invention, the target sequence identified previously, Accession Number AC025249\_C, was subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such suitable sequences were then employed as the forward and reverse primers in a PCR amplification based on a library containing a wide range of cDNA species. The resulting amplicon was gel purified, cloned and sequenced to high redundancy to provide the sequence reported below, which is designated Accession Number AC025249\_C1. There are 13 bases that are different between the two sequences.

The disclosed novel GPCR8c nucleic acid of 985 nucleotides (also referred to as AC025249\_C1) is shown in Table 8F. An open reading frame begins with an ATG initiation codon at nucleotides 5-7 and ends with a TGA codon at nucleotides 977-979. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 8F, and the start and stop codons are in bold letters.

**Table 8F. GPCR8c Nucleotide Sequence (SEQ ID NO:35)**

TGTGATGCTGGGTCCAGCTTATAACCACACAATGGAACCCCTGCCTCCTTCCTCCTTGTGGGTATCCCAGGACTG  
 CAATCTTCACATCTTTGGCTGGCTATCTCACTGAGTGCCATGTACATCACAGCCCTGTTAGGAAACACCATCATCG  
 TGA CTGCAATCTGGATGGATTCCACTCGGCATGAGCCCATGTATTGCTTTCTGTGTGTTCTGGCTGCTGTGGACAT  
 TGT TATGGCCTCCTCGGTGGTACCCAAGATGGTGAGCATCTTCTGCTCAGGAGACAGCTCAATCAGCTTTAGTGCT  
 TGT TTTCACTCAGATGTTTTTTGTCCACTTAGCCACAGCTGTGGAGACGGGGCTGCTGCTGACCATGGCTTTTGACC  
 GCTATGTAGCCATCTGCAAGCCTCTACACTACAAGAGAATTCTCACGCCTCAAGTGATGCTGGGAATGAGTATGGC  
 CATCACCATCAGAGCTATCATAGCCATAACTCCACTGAGTTGGATGGTGAGTCATCTACCTTTCTGTGGCTCCAAT  
 GTGGTTGTCCACTCCTACTGTGAGCACATAGCTTTGGCCAGGTTAGCATGTGCTGACCCCGTGCCAGCAGTCTCT  
 ACAGTCTGATTGGTTCTCTCTTATGGTGGGCTCTGATGTGGCCTTCATTGCTGCCTCCTATATCTTAATTCTCAG  
 GGCAGTATTTGATCTCTCCTCAAAGACTGCTCAGTTGAAAGCATTAAAGCACATGTGGCTCCCATGTGGGGTTATG  
 GCTTTGTACTATCTACCTGGGATGGCATCCATCTATGCGGCCTGGTTGGGGCAGGATATAGTGCCTTCACACCCC  
 AAGTGTCTTAGCTGACCTGTACGTGATCATCCAGCCACTTAAATCCCATCATCTATGGCATGAGGACCAACA  
 ATTGCTGGAGGGAATATGGAGTTATCTGATGCACTTCTCTTTGACCACTCCAACCTGGGTTCATGAACACAA

The GPCR8c protein encoded by SEQ ID NO:35 has 324 amino acid residues, and is presented using the one-letter code in Table 8G (SEQ ID NO:36). The SignalP, Psort and/or Hydropathy profile for GPCR8c predict that GPCR8c has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence with a cleavage site at the slash in the sequence VTA-IW, between amino acids 52 and 53. This is typical of this type of membrane protein. The molecular weight of the GPCR8c protein is 35273.4 Daltons.

**Table 8G. Encoded GPCR8c protein sequence (SEQ ID NO:36).**

MLGPAYNHTMETPASFLLVGIPGLQSSHLWLAI SLSAMYITALLGNTIIVTAIWMDSTRHEPMYCFCLVLA AVDIV  
 MASSVVPKMVSIFCSGDSSI SFSACFTQMEFFVHLATAVETGLLLTMAFDRYVAICKPLHYKRILTPOV MLGMSMAI  
 TIRAI IAITPLSWMVSHLPFCGSNVVHSYCEHIALARLACADVPSSLYSLIGSSLMVGS DVA FIAASYILILRA  
 VFDLSSKTAQLKALSTCGSHVGMALYYLPGMASIYAAWLGDIVPLHTQVLLADLYVII PATLNP I IYGMRTKQL  
 LEGIWSYLMHFLFDHSNLGS

The full amino acid sequence of the protein of the invention was found to have 146 of 306 amino acid residues (47%) identical to, and 202 of 306 residues (66%) positive with, the 321 amino acid residue Odorant Receptor S18 from *Mus musculus* (ptrn:SPTREMBL-ACC:Q9WU89) (E value =  $6.8e^{-73}$ ), 130 of 303 amino acid residues (42%) identical to, and 195 of 303 residues (64%) positive with, the 326 amino acid residue MOR 3'BETA1 from *Mus musculus* (SPTREMBL-ACC:Q9WVD9) (E value =  $2.1e^{-69}$ ), and 107 of 297 amino acid residues (36%) identical to, and 175 of 297 residues (58%) positive with, the 321 amino acid residue HOR 5'BETA3 from *Homo sapiens* (SPTREMBL-ACC:Q9Y5P1) (E value =  $4.0e^{-52}$ )

**GPCR8d**

A novel nucleic acid was identified by exon linking. The disclosed novel GPCR8d nucleic acid of 981 nucleotides (also referred to as CG53845-02) is shown in Table 8H. An open reading frame begins with an ATG initiation codon at nucleotides 3-5 and ends with a TGA codon at nucleotides 954-956. A putative untranslated region upstream from the

initiation codon and downstream from the termination codon are underlined in Table 8G, and the start and stop codons are in bold letters.

**Table 8H. GPCR8e Nucleotide Sequence (SEQ ID NO:80)**

TGATGCTGGGTCCAGCTTATAACCACACAATGGAAACCCCTGCCTCCTTCCTTGTGGGTATCCCAGGACTGCA  
ATCTTCACATCTTTGGCTGGCTATCTCACTGAGTGCCATGTACATCATAGCCCTGTTAGGAAACACCCTCATCGTG  
ACTGCAATCTGGATGGATTCCACTCGGCATGAGCCCATGTATTGCTTTCTGTGTGTTCTGGCTGCTGTGGACATTG  
TTATGGCCTCCTCCGTGGTACCAAGATGGTGAGCATCTTCTGCTCGGGAGACAGCTCCATCAGCTTTAGTGCTTG  
TTTCACTCAGATGTTTTTTGTCCACTTAGCCACAGCTGTGGAGACGGGGCTGCTGCTGACCATGGCTTTTGACCGC  
TATGTAGCCATCTGCAAGCCTCTACACTACAAGAGAATTCTCACGCCTCAAGTGATGCTGGGAATGAGTATGGCCG  
TCACCATCAGAGCTGTACATTCATGACTCCACTGAGTTGGATGATGAATCATCTACCTTTCTGTGGCTCCAATGT  
GGTTGTCCACTCCTACTGTAAGCACATAGCTTTGGCCAGGTTAGCATGTGCTGACCCCGTGCCAGCAGTCTCTAC  
AGTCTGATTGGTTCTCTTATGGTGGGCTCTGATGTGGCCTTCATTGCTGCCTCCTATATCTTAATTTCTAGGG  
CAGTATTTGATCTCTCCTCAAAGACTGCTCAGTTGAAAGCATTAAAGCACATGTGGCTCCCATGTGGGGGTTATGGC  
TTTGTACTATCTACTGGGATGGCATCCATCTATGCGGCCCTGGTTGGGGCAGGATATAGTGCCTTGACACCCAA  
GTGCTGCTAGCTGACCTGTACGTGATCATCCAGCCACTTTAAATCCCATCATCTATGGCATGAGGACCAACAAT  
TGCTGGAGGAATATGGAGTTATCTGATGCACTGTCTCTTTGACCACTCCAACCTGGGTTTCATGAACA

- 5 The disclosed nucleic acid sequence has 538 of 858 bases (62%) identical to a *Mus musculus* odorant receptor S19 gene, complete cds:(GENBANK-ID:AF121976|acc:AF121976) (E value =  $3.5e^{-41}$ ).

- The GPCR8d protein encoded by SEQ ID NO:80 has 317 amino acid residues, and is presented using the one-letter code in Table 8I (SEQ ID NO:81). The SignalP, Psort and/or  
10 Hydropathy profile for GPCR8d predict that GPCR8d has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence with a cleavage site at the slash in the sequence VTA-IW, between amino acids 52 and 53. This is typical of this type of membrane protein.

**Table 8I. Encoded GPCR8d protein sequence (SEQ ID NO:81).**

MLGPAYNHTMETPASFLLVGIPGLQSSHLWLAISSLAMYIIALLGNTLIVTAIWMDSTRHEPMYCFLCVLAAVDIV  
MASSVVPKMVSIFSGDSSISFSACFTQMFFVHLATAVETGLLLTMAFDYVAICKPLHYKRIILTPQVLMGSMVA  
TIRAVTFMTPLSWMMNHLPCGNSVVVHSYCKHIALARLACADVPSSLYSLIGSSLMVGSVDVAFIAASYILILRA  
VFDLSSKTAQLKALSTCGSHVGMALYYLPGMASIYAAWLQDIVPLHTQVLLADLYVVIIPATLNPIIYGMRTKQL  
LEGIWSYLMHCPL

15

The full amino acid sequence of the protein of the invention was found to have 142 of 308 amino acid residues (46%) identical to, and 202 of 308 residues (65%) positive with, the 321 amino acid residue Odorant Receptor S18 from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU89) (E value =  $1.5e^{-71}$ )

- 20 GPCR8d is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and

umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, MHC II and III expressing cells, nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources..

### GPCR8e

A novel nucleic acid was identified by exon linking. The disclosed novel GPCR8e nucleic acid of 982 nucleotides (also referred to as CG53845-03) is shown in Table 8J. An open reading frame begins with an ATG initiation codon at nucleotides 4-6 and ends with a TGA codon at nucleotides 978-980. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 8J, and the start and stop codons are in bold letters.

**Table 8J. GPCR8e Nucleotide Sequence (SEQ ID NO:82)**

TTGATGCTGGGTCCAGCTTACAACCACACAATGGAAACCCCTGCCTCCTTCCCTTGTGGGTATCCAGGACTGC  
AATCTTCACATCTTTGGCTGGCTATCTCACTGAGTGCCATGTACATCATAGCCCTGTAGGAAACACCCCTCATCGT  
GACTGCAATCTGGATGGATTCCACTCGGCATGAGCCCATGTATTGCTTTCTGTGTGTTCTGGCTGCTGTGGACATT  
GTTATGGCCTCCTCGTGGTACCCAGATGGTGAGCATCTTCTGCTCGGGAGACAGCTCCATCAGCTTTAGTGCTT  
GTTTCACTCAGATGTTTTTGTCCACTTAGCCACAGCTGTGGAGACGGGGCTGCTGCTGACCATGGCTTTTGACCG  
CTATGTAGCCATCTGCAAGCCTCTACACTACAAGAGAATTCTCAGCCTCAAGTGATGCTGGGAATGAGTATGGCC  
GTCACCATCAGAGCTGTACATTCATGACTCCACTGAGTTGGATGATGAATCATCTACCTTTCTGTGGCTCCAATG  
TGGTTGTCCACTCCTACTGTAAGCACATAGCTTTGGCCAGGTTAGCATGTGCTGACCCCGTGCCAGCAGCCTCTA  
CAGTCTGATTGGTTCTCTTATGGTGGGCTCTGATGTGGCCTTCATTGCTGCCTCCTATATCTTAATCTCAGG  
GCAGTATTGATCTCTCCTCAAAGACTGCTCAGTTGAAAGCATTAAAGCACATGTGGCTCCCATGTGGGGTTATGG  
CTTTGTACTATCTACCTGGGATGGCATCCATCTATGCGGCCTGGTTGGGGCAGGATATAGTGCCCTTGACACCCA  
AGTGCTGCTAGCTGACCTGTACGTGATCATCCAGCCACTTTAAATCCCATCATCTATGGCATGAGGACCAACAA  
TTGCTGGAGGAATATGGAGTTATCTGATGCACTTCTCTTGACCACTCCAACCTGGGTTTCATGAACAA

The disclosed nucleic acid sequence has 538 of 858 bases (62%) identical to a *Mus musculus* odorant receptor S19 gene, complete cds:(GENBANK- ID:AF121976|acc:AF121976) (E value =  $4.5e^{-42}$ ).

The GPCR8e protein encoded by SEQ ID NO:82 has 324 amino acid residues, and is presented using the one-letter code in Table 8K (SEQ ID NO:83). The SignalP, Psort and/or Hydropathy profile for GPCR8e predict that GPCR8e has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. The SignalP shows a signal sequence with a cleavage site at the slash in the sequence VTA-IW, between amino acids 52 and 53. This is typical of this type of membrane protein.

**Table 8K. Encoded GPCR8d protein sequence (SEQ ID NO:81).**

MLGPAYNHTMETPASFLLVGIPGLQSSHLWLAISSLSAMYIIALLGNTLIVTAIWMDSRHEPMYCFLCVLAAVDIV  
 MASSVVPKMVSIFCSGDSSISFSACFTQMFVHLATAVETGLLLTMAFDYVAICKPLHYKRILTQVMLGMSMAV  
 TIRAVTFMTPLSWMMNHLPCGSGNVVHSHYCKHIALARLACADVPSSLYSLIGSSLMVGSDDVAFTAAASYILILRA  
 VFDLSSKTAQLKALSTCGSHVGMALYYLPGMASIYAAWLGQDIVPLHTQVLLADLYVIIPATLNPIIYGMRTKQL  
 LEGIWSYLMHFLFDHNSNLGS

The full amino acid sequence of the protein of the invention was found to have 142 of 308 amino acid residues (46%) identical to, and 202 of 308 residues (65%) positive with, the 321 amino acid residue Odorant Receptor S18 from *Mus musculus* (ptnr:SPTREMBL-ACC:Q9WU89) (E value =  $1.5e^{-71}$ )

GPCR8e is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, MHC II and III expressing cells, nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. Expression information was derived from the tissue sources of the sequences that were included in the derivation of the sequence of CuraGen Acc. No. CG53845-03.

Further BLAST analysis produced the significant results listed in Table 8L. The disclosed GPCR8 protein has good identity with a number of olfactory receptor proteins.

**Table 8L. BLAST results for GPCR8**

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 9938014 ref NP_064686.1	Odorant receptor S18 gene [Mus musculus]	321	142/308 (46%)	202/308 (65%)	2e-65
gi 11908211 gb AAG41676.1  (AF137396)	HOR 5'Beta14 [Homo sapiens]	318	134/292 (45%)	187/292 (63%)	2e-64
gi 6532001 gb AAD27596.2 AF121976_1 (AF121976)	Odorant receptor S19 [Mus musculus]	339	128/281 (45%)	185/281 (65%)	1e-63
gi 7305349 ref NP_038647.1	Olfactory receptor 67 [Mus musculus]	326	129/299 (43%)	192/299 (64%)	3e-62
gi 11875778 gb AAG40776.1 AF311306_1 (AF311306)	Prostate specific G-protein coupled receptor; PSGR [Homo sapiens]	320	124/293 (42%)	190/293 (64%)	1e-60

This information is presented graphically in the multiple sequence alignment given in Table 8M (with GPCR8 being shown on line 1) as a ClustalW analysis comparing GPCR8 with related protein sequences.

5 Table 8M. Information for the ClustalW proteins:

- 1) GPCR8a (SEQ ID NO:32)  
 2) GPCR8b (SEQ ID NO:34)  
 3) GPCR8c (SEQ ID NO:36)  
 10 2) gi|9938014|ref|NP\_064686.1| odorant receptor S18 gene [Mus musculus] (SEQ ID NO:56)  
 3) gi|11908211|gb|AAG41676.1| (AF137396) HOR 5'Beta14 [Homo sapiens] (SEQ ID NO:55)  
 4) gi|6532001|gb|AAD27596.2|AF121976\_1 (AF121976) odorant receptor S19 [Mus musculus] (SEQ ID NO:54)  
 5) gi|7305349|ref|NP\_038647.1| olfactory receptor 67 [Mus musculus] (SEQ ID NO:57)  
 15 6) gi|11875778|gb|AAG40776.1|AF311306\_1 (AF311306) prostate specific G-protein coupled (SEQ ID NO:65)

	10	20	30	40	50	60
GPCR8a	.....	.....	.....	.....	.....	.....
GPCR8b	.....	.....	.....	.....	.....	.....
GPCR8c	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 11875778	.....	.....	.....	.....	.....	.....
	70	80	90	100	110	120
GPCR8a	.....	.....	.....	.....	.....	.....
GPCR8b	.....	.....	.....	.....	.....	.....
GPCR8c	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 11875778	.....	.....	.....	.....	.....	.....
	130	140	150	160	170	180
GPCR8a	.....	.....	.....	.....	.....	.....
GPCR8b	.....	.....	.....	.....	.....	.....
GPCR8c	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 11875778	.....	.....	.....	.....	.....	.....
	190	200	210	220	230	240
GPCR8a	.....	.....	.....	.....	.....	.....
GPCR8b	.....	.....	.....	.....	.....	.....
GPCR8c	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....
gi 7305349	.....	.....	.....	.....	.....	.....
gi 11875778	.....	.....	.....	.....	.....	.....
	250	260	270	280	290	300
GPCR8a	.....	.....	.....	.....	.....	.....
GPCR8b	.....	.....	.....	.....	.....	.....
GPCR8c	.....	.....	.....	.....	.....	.....
gi 9938014	.....	.....	.....	.....	.....	.....
gi 11908211	.....	.....	.....	.....	.....	.....
gi 6532001	.....	.....	.....	.....	.....	.....

gi 7305349	LIITSYTHLIRTVFQIPSTANFYKAIATCGSHICVILLFYTPAFFSFEAHRFGGKTVPRH
gi 11875778	FTLSYFLIRTVFQIPSTANFYKAIATCGSHICVILLFYTPAFFSFEAHRFGGKTVPRH
	310 320 330 340 350
GPCR8a	TOVLLADLYVETPATLNPIIYGMTKQILEGWSYDMHFLFDHNLGS--
GPCR8b	TOVLLADLYVETPATLNPIIYGMTKQILEGWSYDMHFLFDHNLGS--
GPCR8c	TOVLLADLYVETPATLNPIIYGMTKQILEGWSYDMHFLFDHNLGS--
gi 9938014	HHLLANLYVVPPELNPIIYGATKQIRDSITRMISVVWKS-----
gi 11908211	VHDSLANLYVVPPELNPIIYGATKQIRDSITRMISVVWKS-----
gi 6532001	VHDSLANLYVVPPELNPIIYGATKQIRDSITRMISVVWKS-----
gi 7305349	HHLLANLYVVPPELNPIIYGATKQIRDSITRMISVVWKS-----
gi 11875778	HHLLANLYVVPPELNPIIYGATKQIRDSITRMISVVWKS-----

The presence of identifiable domains in GPCR8 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR8 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 8N with the statistics and domain description. The results indicate that GPCR8 has homology to the 7tm\_1 (InterPro) 7 transmembrane receptor (rhodopsin family) domain (as defined by Interpro). This indicates that the sequence of GPCR8 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

**Table 8N. DOMAIN results for GPCR8 against the consensus 7tm**

**1 domain (SEQ ID NO:78)**

CD-Length = 254 residues, 100.0% aligned  
Score = 79.7 bits (195), Expect = 2e-16

		10	20	30	40	50	60
GPCR8 (SEQ ID NO:32)	.....	.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain	.....	.....	.....	.....	.....	.....	.....
		70	80	90	100	110	120
GPCR8	.....	.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain	.....	.....	.....	.....	.....	.....	.....
		130	140	150	160	170	180
GPCR8	.....	.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain	.....	.....	.....	.....	.....	.....	.....
		190	200	210	220	230	240
GPCR8	.....	.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain	.....	.....	.....	.....	.....	.....	.....
		250	260	270	280	290	300
GPCR8	.....	.....	.....	.....	.....	.....	.....
Consensus 7tm 1 domain	.....	.....	.....	.....	.....	.....	.....
		310	320	330	340	350	360



5

10 The similarity information for the GPCR8 protein and nucleic acid disclosed herein suggest that GPCR8 may have important structural and/or physiological functions characteristic of the Olfactory Receptor family and the GPCR family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo (vi) biological defense weapon. The novel nucleic acid encoding GPCR8, and the GPCR8 protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

The disclosed GPCR8 nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus

cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR Antibodies" section below. For example the disclosed GPCR8 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR8 epitope is from about amino acids 50 to 60. In another embodiment, a GPCR8 epitope is from about amino acids 130 to 140. In additional embodiments, GPCR8 epitopes are from amino acids 230 to 240, and 280-310. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

#### GPCR9

##### GPCR9a

A novel nucleic acid was identified on chromosome 11 by TblastN using CuraGen Corporation's sequence file for GPCR probe or homolog, run against the Genomic Daily Files made available by GenBank. The nucleic acid was further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length protein. The disclosed novel GPCR9a nucleic acid of 960 nucleotides (also referred to as AC025249\_D) is shown in Table 9A. An open reading frame begins with an ATG initiation codon at nucleotides 4-6 and ends with a TAG codon at nucleotides 951-953. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 9A, and the start and stop codons are in bold letters.

The nucleic acid sequence has 567 of 880 bases (64%) identical to ( $E = 2.6e^{-53}$ ) a G-protein coupled prostate tissue receptor designated HPRAJ70 cDNA from *Homo sapiens* (patn:V30143).

**Table 9A. GPCR9a Nucleotide Sequence (SEQ ID NO:37)**

```
GCCATGCTCACTTTTCATAATGTCTGCTCAGTACCCAGCTCCTTCTGGCTCACTGGCATCCCAGGGCTGGA
GTCCCTACACGTCTGGCTCTCCATCCCCTTTGGCTCCATGTACCTGGTGGCTGTGGTGGGGAATGTGACCA
TCCTGGCTGTGGTAAAGATAGAACGCAGCCTGCACCAGCCCATGTACTTTTCTTGTGCATGTTGGCTGCC
ATTGACCTGGTTCTGTCTACTTCCACTATACCCAACTTCTGGGAATCTTCTGGTTCGGTGCTTGTGACAT
TGGCCTGGACGCCTGCTTGGGCCAAATGTTCTTATCCACTGCTTTGCCACTGTTGAGTCAGGCATCTTCC
TTGCCATGGCTTTTGATCGCTACGTGGCCATCTGCAACCCACTACGTCATAGCATGGTGCTCACTTATACA
GTGGTGGGTCGTTTGGGGCTTGTCTCTCTCCTCGGGGTGTTCTCTACATTGGACCTCTGCCTCTGATGAT
CCGCCTGCGGCTGCCCTTTATAAAACCCATGTTATCTCCCACTCCTACTGTGAGCACATGGCTGTAGTTG
CCTTGACATGTGGCGACAGCAGGGTCAATAATGTCTATGGGCTGAGCATCGGCTTCTGGTGTGATCCTG
GACTCAGTGGCTATTGCTGCATCCTATGTGATGATTTTCAGGGCCGTGATGGGGTTAGCCACTCCTGAGGC
TAGGCTTAAACCCCTGGGGACATGCGCTTCTCACCTCTGTGCCATCCTGATCTTTATGTTCCTATGCTG
TTTCTTCCCTGATTACCGATTGGTCACTGTGTGCCTCCTCCAGTCCACACTCTGCTGGCCAACTTCTAT
CTCCTCATTCCTCAATCTCAATCCCATTTGTCTATGCTGTTCGCACCAAGCAGATCCGAGAGAGCCTTCT
CCAAATACCAAGGATAGAAATGAAGATTAGATGATTA
```

5

The GPCR9a protein encoded by SEQ ID NO:37 has 317 amino acid residues, and is presented using the one-letter code in Table 9B (SEQ ID NO:38). The SignalP, Psort and/or Hydropathy profile for GPCR9a predict that GPCR9a has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The SignalP predicts a cleavage site at the sequence VVG-NV between amino acids 43 and 44. The Molecular weight of GPCR9a is 35036.8 Daltons.

10

**Table 9B. Encoded GPCR9 protein sequence (SEQ ID NO:38)**

```
MLTFHNVCSVPSSFWLTGIPGLES LHVWLSIPFGSMYLVAVVGNVTILAVVKIERSLHQPMYFFLCMLAAI
DLVLSTSTIPKLLGIFWFGACDIGLDACLGQMF L IHC FATVESGIFLAMAEDRYVAICNPLRHSMLVITYV
VGRGLVLSLLRGVLYIGPLPLMIRLRLPLYKTHVISHSYCEHMAVVALTCGDSRVNNVYGLSIGFLVLILD
SVAIAASYVMI FRAVMGLATPEARLKT LGTCASHLCAILIFVVP IAVSSLIHRFGQCVFPFVHTLLANFYL
LIPPILNP I VYAVRTKQ IRESLLQIPRIEMKIR
```

The full amino acid sequence of the protein of the invention was found to have 157 of 301 amino acid residues (52%) identical to, and 210 of 301 residues (69%) positive with, the 320 amino acid residue Putative G-Protein Coupled Receptor RA1C from *Rattus norvegicus* (ptnr:SP TREMBL-ACC:O88628) ( $E$  value =  $2.7e^{-81}$ ).

15

GPCR9 also has homology to the proteins shown in the BLAST alignments in Table 9C.

Table 9C. BLASTX results for GPCR9a

Sequences producing High-scoring Segment Pairs:	Reading Frame	High Score	Smallest Sum Prob P(N)	N
patp:Y92365 G protein-coupled receptor protein 5 - Hom.	+1	831	4.0e-82	1
patp:W01730 Human G-protein receptor HPRAJ70 - Homo sa.	+1	802	4.7e-79	1
patp:W56641 G-protein coupled prostate tissue receptor.	+1	802	4.7e-79	1
patp:R27869 Odorant receptor clone F6 - Rattus rattus,	+1	418	2.3e-38	1

**GPCR9b**

In the present invention, the target sequence identified previously, Accession Number AC079759\_D, was subjected to the exon linking process to confirm the sequence. PCR

5 primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such primers were

10 designed based on in silico predictions for the full length cDNA, part (one or more exons) of the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related human sequences sequences from other species. These primers were then employed in PCR amplification based on the following pool of human cDNAs: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain -

15 hippocampus, brain - substantia nigra, brain - thalamus, brain -whole, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. Usually the resulting amplicons were gel purified, cloned and sequenced to high redundancy. The resulting sequences from all clones were

20 assembled with themselves, with other fragments in CuraGen Corporation's database and with public ESTs. Fragments and ESTs were included as components for an assembly when the extent of their identity with another component of the assembly was at least 95% over 50 bp. In addition, sequence traces were evaluated manually and edited for corrections if appropriate. These procedures provide the sequence reported below, which is designated Accession

25 Number AC079759\_da1. This differs from the previously identified sequence (Accession Number AC079759\_D) in having a single aminoacid change.

The disclosed novel GPCR9b nucleic acid of 997 nucleotides (also referred to as AC025249\_da1) is shown in Table 9D. An open reading frame begins with an ATG initiation codon at nucleotides 5-7 and ends with a TGA codon at nucleotides 956-958. A putative

untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 9D, and the start and stop codons are in bold letters.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 587 of 896 bases (65%) identical to (E= 2.7e-53) a *Rattus norvegicus* putative G-protein coupled receptor RA1c mRNA, complete cds (GENBANK-ID:AF079864|acc:AF079864.1).

**Table 9D. GPCR9b Nucleotide Sequence (SEQ ID NO:39)**

AGCCATGCTCACTTTTCATAATGTCGCTCAGTACCCAGCTCCTTCTGGCTCACTGGCATCCCAGGGCTGG  
 AGTCCCTACACGTCCTGGCTCTCCATCCCCTTTGGCTCCATGTACCTGGTGGCTGTGGTGGGGAATGTGACC  
 ATCCTGGCTGTGGTAAAGATAGAACGCAGCCTGCACCAGCCCATGTACTTTTCTTGTGCATGTTGGCTGC  
 CATTGACCTGGTCTCTACTTCCACTATACCCAACTTCTGGGAATCTTCTGGTTCGGTCTGTTGACA  
 TTGGCCTGGATGCCCTGCTGGGCCAAATGTTCTTATCCACTGCTTTGCCACTGTTGAGTCAGGCATCTTC  
 CTGCGCATGGCTTTTGATCGCTATGTGGCCATCTGCAACCCACTACGTATAGCATGGTGCTCACTATAC  
 AGTGGTGGGTGCTTTGGGGCTTGTCTCTCCTCCGGGTGTTCTCTACATTGGACCTCTGCCTCTGATGA  
 TCCGCTGCGGCTGCCCCCTTATAAAACCCATGTTATCTCCCACTCTACTGTGAGCACATGGCTGTAGTT  
 GCCTTGACATGTGGCGACAGCAGGTCATAATGTCTATGGGCTGAGCATCGGCTTTCTGGTGTGATCCT  
 GGACTCAGTGGCTATTGCTGCATCCTATGTGATGATTTTCAGGGCCGTGATGGGGTTAGCCACTCCTGAGG  
 CTAGGCTTAAACCCCTGGGACATGCGCTTCTCACCTCTGTGCCATCCTGATCTTTATGTTCCCATTTGCT  
 GTTTCTTCCCTGATTCACCGATTTGGTCAGTGTGTGCCTCCTCCAGTCCCACTCTGCTGGCCAACTTCTA  
 TCTCCTCATTCCTCCAATCCTCAATCCCATTTGTCTATGCTGTTCCGACCAAGCAGATCCGAGAGAGGCTTC  
 TCCAAATACCAAGGATAGAAATGAAGATTAGATGATTACTATTTTCTTCTCTCAAATAAGCTCATGGAG  
AAG

The GPCR9b protein encoded by SEQ ID NO:39 has 317 amino acid residues, and is presented using the one-letter code in Table 9E (SEQ ID NO:40). The SignalP, Psort and/or Hydropathy profile for GPCR9 predict that GPCR9 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The SignalP predicts a cleavage site at the sequence VVG-NV between amino acids 43 and 44.

**Table 9E. Encoded GPCR9b protein sequence (SEQ ID NO:40)**

MLTFHNVCSPVSSFWLTGIPGLESLSHVWLSIPFGSMYLVAVVGNVTILAVVKIERSLHQPMYFFLCMLAAI  
 DLVLSTSTIPKLLGIFWFGACDIGLDACLGQMFILHCFATVESGIFLAMAFDRYVAICNPLRHSMLVITYV  
 VGRGLVLSLLRGVLYIGPLPLMIRLRLPLYKTHVISHSYCEHMAVVALTCGDSRVNNVYGLSIGFLVLILD  
 SVAIAASYVMI FRAVMGLATPEARLKTGT CASHLCAILIFYVPIAVSSLIHRFGQCVPPFVHTLLANFYL  
 LI PPI LNPIVYAVRTKQIRERLLQIPRIEMKIR

The full amino acid sequence of the protein of the invention was found to have 158 of 301 amino acid residues (52%) identical to, and 211 of 301 residues (70%) positive with, the 320 amino acid residue Putative G-Protein Coupled Receptor RA1C from *Rattus norvegicus* (ptnr:SPTREMBL-ACC:O88628) (E value = 2.7e-<sup>82</sup>)

The Olfactory Receptor disclosed in this invention is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate

nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, MHC  
5 II abnd III expressing cells, nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to  
10 SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources.

### GPCR9c

In the present invention, the target sequence identified previously, Accession Number AC079759\_D, was subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward  
15 primer, and at the most downstream sequence available for the reverse primer. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such primers were designed based on in silico predictions for the full length cDNA, part (one or more exons) of  
20 the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related human sequences sequences from other species. These primers were then employed in PCR amplification based on the following pool of human cDNAs: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain - hippocampus, brain - substantia nigra, brain - thalamus, brain -whole, fetal brain, fetal kidney,  
25 fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. Usually the resulting amplicons were gel purified, cloned and sequenced to high redundancy. The resulting sequences from all clones were assembled with themselves, with other fragments in CuraGen Corporation's database and with  
30 public ESTs. Fragments and ESTs were included as components for an assembly when the extent of their identity with another component of the assembly was at least 95% over 50 bp. In addition, sequence traces were evaluated manually and edited for corrections if appropriate. These procedures provide the sequence reported below, which is designated Accession

Number AC079759\_da1. This differs from the previously identified sequence (Accession Number AC079759\_D) in having a single aminoacid change.

The disclosed novel GPCR9c nucleic acid of 997 nucleotides (also referred to as CG50223-01) is shown in Table 9F An open reading frame begins with an ATG initiation  
 5 codon at nucleotides 5-7 and ends with a TGA codon at nucleotides 958-960. A putative untranslated region upstream from the initiation codon and downstream from the termination codon are underlined in Table 9F, and the start and stop codons are in bold letters.

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 573 of 891 bases (64%) identical to (E= 3.7e-52) a Sequence 1 from patent US  
 10 5756309 (GENBANK-ID:AR009514|acc:AR009514.1).

**Table 9F. GPCR9c Nucleotide Sequence (SEQ ID NO:41)**

```
AGCCATGCTCACTTTTCATAATGTCTGCTCAGTACCCAGCTCCTTCTGGCTCACTGGCATCCCAGGGCTGG
AGTCCCTACACGTCTGGCTCTCCATCCCCTTTGGCTCCATGTACCTGGTGGCTGTGGTGGGAATGTGACC
ATCCTGGCTGTGGTAAAGATAGAACGCAGCCTGCACCAGCCCATGTACTTTTCTTGTCATGTTGGCTGC
CATTGACCTGGTTCTGTCTACTTCCACTATACCCAACTTCTGGGAATCTTCTGGTTCGGTGCTTGTGACA
TTGGCCTGGATGCCTGCTTGGGCCAAATGTTCTTATCCACTGCTTTGCCACTGTTGAGTCAGGCATCTTC
CTTGCCATGGCTTTTGATCGCTACGTGGCCATCTGCAACCCACTACGTCATAGCATGGTGCTCACTTATAC
AGTGGTGGGTCGTTTGGGGCTTGTCTCTCCTCCGGGGTGTCTCTACATTGGACCTCTGCCTCTGATGA
TCCGCCTGCGGCTGCCCTTTATAAAACCCATGTTATCTCCCACTCCTACTGTGAGCACATGGCTGAGTT
GCCTTGACATGTGGCGACAGCAGGGTCAATAATGTCTATGGGCTGAGCATCGGCTTTCTGGTGTGATCCT
GGACTCAGTGGCTATTGCTGCATCCTATGTGATGATTTTCAGGGCCGTGATGGGGTTAGCCACTCCTGAGG
CTAGGCTTAAACCCCTGGGGACATGCGCTTCTCACTCTGTGCCATCCTGATCTTTTATGTTCCCATTTGCT
GTTTCTTCCCTGATTACCGATTGGTCACTGTGTGCCTCTCCAGTCCACACTCTGCTGGCCAACCTCTTA
TCTCCTCATTCTCCAATCCTCAATCCCATTTGTCTATGCTGTTTCGCACCAAGCAGATCCGAGAGAGGCTTC
TCCAATAACCAAGGATAGAAATGAAGATTAGATGATTACTATTTTCTCTCTCTCAAATAAGCTCATGGAG
AAG
```

The GPCR9c protein encoded by SEQ ID NO:41 has 317 amino acid residues, and is presented using the one-letter code in Table 9G (SEQ ID NO:42). The SignalP, Psort and/or  
 15 Hydropathy profile for GPCR9 predict that GPCR9 has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6000. The SignalP predicts a cleavage site at the sequence VVG-NV between amino acids 43 and 44.

**Table 9G. Encoded GPCR9c protein sequence (SEQ ID NO:42)**

```
MLTFHNVCVSPSSFWLTGIPGLESLSLHVLSIPFGSMYLVAVVGNVTILAVVKIERSLHQPMYFFLCMLAAI
DLVLSTSTIPKLLGIFWFGACDGLDACLGMFLIHCFAVESGIFLAMAFDRYVAICNPLRHSMLVITYTV
VGRGLVLSLLRGVLYIGPLPLMIRLRLPLYKTHVISHSYCEHMAVVALT CGDSGVNNVYGLSIGFLVLILD
SVAIAASYVMI FRAVMGLATPEARLKTLTGCASHLCAILIFYIPIAVSSLIHRFGQCVPVPPVHTLLANFYL
LIPFILNPVIVAVRTKQIRERLLQIPRIEMKIR
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20 The full amino acid sequence of the protein of the invention was found to have 157 of 301 amino acid residues (52%) identical to, and 211 of 301 residues (70%) positive with, the

320 amino acid residue Putative G-Protein Coupled Receptor RA1C from *Rattus norvegicus* (ptrr:SPTREMBL-ACC:O88628) (E value =  $5.6e^{-82}$ )

GPCR9c is expressed in at least the following tissues: Apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, MHC II abnd III expressing cells, nervous, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortic spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources.

Possible SNPs found for GPCR9c are listed in Table 9H.

Table 9H: SNPs			
Consensus Position	Depth	Base Change	PAF
298	18	C>T	0.333
445	22	G>A	0.091

Further BLAST analysis produced the significant results listed in Table 9I. The disclosed GPCR9 protein has good identity with a number of olfactory receptor proteins.

Table 9I. BLAST results for GPCR9					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi 11991867 gb AAG42368.1  (AF289204)	odorant receptor HOR3'beta5 [Homo sapiens]	316	154/309 (49%)	193/309 (61%)	2e-71
gi 11908221 gb AAG41685.1  (AF133300)	MOR 3'Beta6 [Mus musculus]	316	151/299 (50%)	189/299 (62%)	3e-70
gi 7305351 ref NP_038648.1	Olfactory receptor 68 [Mus musculus]	315	148/309 (47%)	188/309 (59%)	7e-68
gi 7305353 ref NP_038649.1	Olfactory receptor 69 [Mus musculus]	316	147/309 (47%)	187/309 (59%)	1e-66
gi 3927808 gb AAD03349.1  (L17432)	olfactory receptor-like protein COR3'beta [Gallus gallus]	319	145/296 (48%)	196/296 (65%)	1e-66



This information is presented graphically in the multiple sequence alignment given in Table 9J (with GPCR9a being shown on line 1, GPCR9b on line 2, and GPCR9c on line 3) as a ClustalW analysis comparing GPCR9 with related protein sequences.

Table 9J. Information for the ClustalW proteins:

- 1) GPCR9a (SEQ ID NO:38)
- 2) GPCR9b (SEQ ID NO:40)
- 3) GPCR9c (SEQ ID NO:42)
- 4) gi|11991867|gb|AAG42368.1| (AF289204) odorant receptor HOR3 $\beta$ 5 [Homo sapiens] (SEQ ID NO:66)
- 5) gi|11908221|gb|AAG41685.1| (AF133300) MOR 3 $\beta$ 6 [Mus musculus] (SEQ ID NO:67)
- 6) gi|7305351|ref|NP\_038648.1| olfactory receptor 68 [Mus musculus] (SEQ ID NO:68)
- 7) gi|7305353|ref|NP\_038649.1| olfactory receptor 69 [Mus musculus] (SEQ ID NO:69)
- 8) gi|3927808|gb|AAD03349.1| (L17432) olfactory receptor-like protein COR3 $\beta$  [Gallus gallus] (SEQ ID NO:70)

	10	20	30	40	50	60
GPCR9a	MTFHHVCSVPSSFWLTGIPGLES	HHVMSIPFGSMYLVAVVGNVTILAVV	HERSLHQP			
GPCR9b	MTFHHVCSVPSSFWLTGIPGLES	HHVMSIPFGSMYLVAVVGNVTILAVV	HERSLHQP			
GPCR9c	MTFHHVCSVPSSFWLTGIPGLES	HHVMSIPFGSMYLVAVVGNVTILAVV	HERSLHQP			
gi 11991867	MPTEHGSVFMPASILLIGIPGLES	VOCHGIPFSAMYLIGVIGNSLILV	IKHNSLHQP			
gi 11908221	MPHINSITFRPSVLTLTGIPGLES	VOHAGIPFCINVTANIGNSLILV	IKHNSLHQP			
gi 7305351	MTKFGSVFMPSVLTIVGIPGLES	VOCHGIPFCVMYHIANIGNSLILV	IKHNSLHQP			
gi 7305353	MTKFGSVFMPSVLTIVGIPGLES	VOCHGIPFCVMYHIANIGNSLILV	IKHNSLHQP			
gi 3927808	MYPRNSQAQ--ELIACLPQMAQFH	HHVETPEGLNYIVAVLGNSTILLV	VRVHFOLHQP			
	70	80	90	100	110	120
GPCR9a	MYEFLCMLAAIDLVLTSTTPHLLG	IFWFGACDGLDACLCQELIHCFATV	ESGILLAM			
GPCR9b	MYEFLCMLAAIDLVLTSTTPHLLG	IFWFGACDGLDACLCQELIHCFATV	ESGILLAM			
GPCR9c	MYEFLCMLAAIDLVLTSTTPHLLG	IFWFGACDGLDACLCQELIHCFATV	ESGILLAM			
gi 11991867	MYIFLAMLATDIDLVLTSTTPHLLG	IFWFLPETSFOACLFQMLIESFOAT	ESSGILLAM			
gi 11908221	MYIFLAMLATDIDLVLTSTTPHLLG	IFWFLPETSFOACLFQMLIESFOAT	ESSGILLAM			
gi 7305351	MYIFLAMLATDIDLVLTSTTPHLLG	IFWFLPETSFOACLFQMLIESFOAT	ESSGILLAM			
gi 7305353	MYIFLAMLATDIDLVLTSTTPHLLG	IFWFLPETSFOACLFQMLIESFOAT	ESSGILLAM			
gi 3927808	MYIFLAMLATDIDLVLTSTTPHLLG	IFWFLPETSFOACLFQMLIESFOAT	ESSGILLAM			
	130	140	150	160	170	180
GPCR9a	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
GPCR9b	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
GPCR9c	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
gi 11991867	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
gi 11908221	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
gi 7305351	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
gi 7305353	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
gi 3927808	ADRYVAICNPLRHSMVLTITVYCR	GLVSLRGVLYGCPIDMIRLRPLK	YTHVISHS			
	190	200	210	220	230	240
GPCR9a	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
GPCR9b	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
GPCR9c	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
gi 11991867	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
gi 11908221	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
gi 7305351	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
gi 7305353	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
gi 3927808	YCEHMAVVALTCGDSRVNNVYGL	STGSLVLLDSVAIAASYVMIFRAVM	GLATPEARLKT			
	250	260	270	280	290	300
GPCR9a	LGTCASHLCAILIFVPIAVSSLI	HRFGCCVPPVHTLLANFYLLIP	PLNPIVYAVRTE			
GPCR9b	LGTCASHLCAILIFVPIAVSSLI	HRFGCCVPPVHTLLANFYLLIP	PLNPIVYAVRTE			
GPCR9c	LGTCASHLCAILIFVPIAVSSLI	HRFGCCVPPVHTLLANFYLLIP	PLNPIVYAVRTE			
gi 11991867	ENTCIAHICVLEFVYLLAFFSFF	THREGSHTPPYHLLSNLYLLVP	PELNPVYGVKTE			
gi 11908221	ENTCIAHICVLEFVYLLAFFSFF	THREGSHTPPYHLLSNLYLLVP	PELNPVYGVKTE			
gi 7305351	ENTCIAHICVLEFVYLLAFFSFF	THREGSHTPPYHLLSNLYLLVP	PELNPVYGVKTE			

gi 7305353	ENTCEAHICVFLQEVYLLAFSSFTTHRECAHPPYVHLLSDLYLLVPPSLNPPIVYGLTH
gi 3927808	INTCVSHFFCAVLTEYUPLAGLSIIHPYGRNAPPISHAVNANVYLVPPILNEVTYSMTSP
	310 320
GPCR9a	QIRSELPQIPRIEMKIR-----
GPCR9b	QIRSELPQIPRIEMKIR-----
GPCR9c	QIRSELPQIPRIEMKIR-----
gi 11991867	QIRUHLVKEFFFKVT-----
gi 11908221	QIRDOVSKLLYCNYSY-----
gi 7305351	QIRDOVSKLLFSKPKH-----
gi 7305353	QIRDOVSKLLFSKPKL-----
gi 3927808	ATCKGLERHLCQRAAWPGHAQNC

The presence of identifiable domains in GPCR9 was determined by searches using algorithms such as PROSITE, Blocks, Pfam, ProDomain, Prints and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (<http://www.ebi.ac.uk/interpro/>).

DOMAIN results for GPCR9 were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST. This BLAST samples domains found in the Smart and Pfam collections. The results are listed in Table 9K with the statistics and domain description. The results indicate that GPCR9 has homology to the 7tm\_1 (InterPro) 7 transmembrane receptor (rhodopsin family) domain (as defined by Interpro). This indicates that the sequence of GPCR9 has properties similar to those of other proteins known to contain this/these domain(s) and similar to the properties of these domains.

**Table 9K. DOMAIN results for GPCR9 against the consensus 7tm  
1 domain (SEQ ID NO:79)**

CD-Length = 254 residues, 100.0% aligned  
Score = 63.9 bits (154), Expect = 1e-11

		10	20	30	40	50	60
15	GPCR9 (SEQ ID NO:38)	GNVTYIAVVKIERSH-CEMYFELCMLAIDLSTETIKLIGIFWFGACDIEIDAG					
	Consensus 7tm 1 domain	GNGLAHLVNLRTKRR-TETNIFLNLEAVADIEFLLEPPWALYYLVGSDWVFEDALPKL					
		70	80	90	100	110	120
20	GPCR9	YCOMETIACNAFESGFLAMEFDRYVAICPLEPSMLTYTVVGRGSLVSLRGVLYIG					
	Consensus 7tm 1 domain	YCALGVNCHASHL--LTAKSIDRYATVHPLPERRRTPRRAKVITLVWMLALULSL					
		130	140	150	160	170	180
25	GPCR9	PLPIMIRRLPLKYTHVISHSYCEHM-----AVVALTCGDSRMNNYELSLRGFL					
	Consensus 7tm 1 domain	PPLLFSWLFTVEEGNT--FVCLIDFP-----EESVK--RSYVLSTIVFLPL					
		190	200	210	220	230	240
30	GPCR9	VITIDSVLAASVYVMIFKVMGLATPE-----					
	Consensus 7tm 1 domain	VITLCYTRILRLRKSAQSQRSLKRRSS-----					
		250	260	270	280	290	300
35	GPCR9	-----					
	Consensus 7tm 1 domain	-----					
		310	320	330	340	350	360

10

20

35

autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders.

The polypeptides can be used as immunogens to produce antibodies specific for the invention, and as vaccines. They can also be used to screen for potential agonist and antagonist compounds. For example, a cDNA encoding the GPCR-like protein may be useful in gene therapy, and the GPCR-like protein may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from developmental diseases, MHCII and III diseases (immune diseases), Taste and scent detectability Disorders, Burkitt's lymphoma, Corticoneurogenic disease, Signal Transduction pathway disorders, Retinal diseases including those involving photoreception, Cell Growth rate disorders; Cell Shape disorders, Feeding disorders; control of feeding; potential obesity due to over-eating; potential disorders due to starvation (lack of appetite), noninsulin-dependent diabetes mellitus (NIDDM1), bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2), pain, cancer (including but not limited to Neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer), anorexia, bulimia, asthma, Parkinson's disease, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, Crohn's disease; multiple sclerosis; and Treatment of Albright Hereditary Osteodystrophy, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation. Dentatorubro-pallidolusian atrophy (DRPLA) Hypophosphatemic rickets, autosomal dominant (2) Acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de la Tourette syndrome and/or other pathologies and disorders. The novel nucleic acid encoding GPCR-like protein, and the GPCR-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the novel GPCR9 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-GPCR9 Antibodies" section below. For example the disclosed GPCR9 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, a contemplated GPCR9 epitope is from about amino acids 130 to 180. In another embodiment, a GPCR9 epitope is from about amino acids

280 to 320. These novel proteins can also be used to develop assay system for functional analysis.

A summary of the GPCR<sub>X</sub> nucleic acids and proteins of the invention is provided in

5 Table 10.

**TABLE 10: Summary Of Nucleic Acids And Proteins Of The Invention**

Name	Tables	Clone; Description of Homolog	Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO
GPCR1	1A, 1B, 1D 1E, 1F	GPCR1a: rp11-507n20_A GPCR	1	2
		GPCR1b: 11-507n20_A_dal, GPCR	3	
		GPCR1c: AX011711_dal, GPCR	4	5
GPCR2	2A, 2B	GPCR2: 80250319_EXT, GPCR	6	7
GPCR3	3A, 3B	GPCR3: AC020597, GPCR	8	9
GPCR4	4A, 4B, 4F, 4G 4J	GPCR4a: AC020597_B, GPCR	10	11
		GPCR4b: AC020597B1, GPCR	12	13
		GPCR4c: AC020597B2, GPCR	14	
GPCR5	5A, 5B, 5D, 5E 5F, 5G	GPCR5a: AC020597_C, GPCR	15	16
		GPCR5b: CG53668-02, GPCR	17	18
		GPCR5c: AC020597B_dal, GPCR	19	20
GPCR6	6A, 6B 6D, 6E	GPCR6a: AC020597_D, GPCR	21	22
		GPCR6b: AC020597_D1, GPCR	23	24
GPCR7	7A, 7B 7F, 7G 7I, 7J	GPCR7a: AC020597_A, GPCR	25	26
		GPCR7b: AC020597_A1, GPCR	27	28
		GPCR7c: AC025249_Ada3, GPCR	29	30
GPCR8	8A, 8B 8D, 8E 8F, 8G 8H, 8I 8J, 8K	GPCR8a: AC025249_B, GPCR	31	32
		GPCR8b: AC025249_C, GPCR	33	34
		GPCR8c: AC025249_C1, GPCR	35	36
		GPCR8d: CG53845-02, GPCR	80	81
		GPCR8e: CG53845-03, GPCR	82	83
GPCR9	9A, 9B 9D, 9E 9F, 9G	GPCR9a: AC025249_D, GPCR	37	38
		GPCR9b: AC079759_D, GPCR	39	40
		GPCR9c: CG50223-01, GPCR	41	42

### GPCR<sub>X</sub> Nucleic Acids and Polypeptides

One aspect of the invention pertains to isolated nucleic acid molecules that encode  
 10 GPCR<sub>X</sub> polypeptides or biologically active portions thereof. Also included in the invention  
 are nucleic acid fragments sufficient for use as hybridization probes to identify GPCR<sub>X</sub>-

encoding nucleic acids (*e.g.*, GPCR<sub>X</sub> mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of GPCR<sub>X</sub> nucleic acid molecules. As used herein, the term “nucleic acid molecule” is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An GPCR<sub>X</sub> nucleic acid can encode a mature GPCR<sub>X</sub> polypeptide. As used herein, a “mature” form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full-length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an ORF described herein. The product “mature” form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a “mature” form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an ORF, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a “mature” form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term “probes”, as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or

double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one, which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid.

5 Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated GPCR<sub>X</sub> nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule  
10 in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

15 A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 4, 6,  
20 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 as a hybridization probe, GPCR<sub>X</sub> molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2<sup>nd</sup> Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John  
25 Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore,  
30 oligonucleotides corresponding to GPCR<sub>X</sub> nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a

genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue.

Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the

invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, or a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, or a portion of this nucleotide sequence (e.g., a fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an GPCR polypeptide). A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid



sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of GPCR<sub>X</sub> polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for an GPCR<sub>X</sub> polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human GPCR<sub>X</sub> protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, as well as a polypeptide

possessing GPCR<sub>X</sub> biological activity. Various biological activities of the GPCR<sub>X</sub> proteins are described below.

5 An GPCR<sub>X</sub> polypeptide is encoded by the open reading frame ("ORF") of an GPCR<sub>X</sub> nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good  
10 candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, e.g., a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human GPCR<sub>X</sub> genes allows for the generation of probes and primers designed for use in identifying and/or cloning GPCR<sub>X</sub> homologues in other cell types, e.g. from other tissues, as well as GPCR<sub>X</sub>  
15 homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82; or an anti-sense  
20 strand nucleotide sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82.

Probes based on the human GPCR<sub>X</sub> nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various  
25 embodiments, the probe further comprises a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an GPCR<sub>X</sub> protein, such as by measuring a level of an GPCR<sub>X</sub>-encoding nucleic acid in a sample of cells from a subject e.g., detecting GPCR<sub>X</sub> mRNA levels or determining  
30 whether a genomic GPCR<sub>X</sub> gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an GPCR<sub>X</sub> polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-

active portion of GPCR<sub>X</sub>" can be prepared by isolating a portion SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 that encodes a polypeptide having an GPCR<sub>X</sub> biological activity (the biological activities of the GPCR<sub>X</sub> proteins are described below), expressing the encoded portion of GPCR<sub>X</sub> protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of GPCR<sub>X</sub>.

### GPCR<sub>X</sub> Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 due to degeneracy of the genetic code and thus encode the same GPCR<sub>X</sub> proteins as that encoded by the nucleotide sequences shown in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83.

In addition to the human GPCR<sub>X</sub> nucleotide sequences shown in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the GPCR<sub>X</sub> polypeptides may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the GPCR<sub>X</sub> genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an GPCR<sub>X</sub> protein, preferably a vertebrate GPCR<sub>X</sub> protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the GPCR<sub>X</sub> genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the GPCR<sub>X</sub> polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the GPCR<sub>X</sub> polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding GPCR<sub>X</sub> proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the GPCR<sub>X</sub> cDNAs of the invention can be isolated based on their homology to the human GPCR<sub>X</sub> nucleic acids disclosed herein using the human

cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding GPCR proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5 °C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at  $T_m$ , 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y.

(1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* 78: 6789-6792.

### Conservative Mutations

In addition to naturally-occurring allelic variants of GPCR<sub>X</sub> sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 thereby leading to changes in the amino acid sequences of the encoded GPCR<sub>X</sub> proteins, without altering the functional ability of said GPCR<sub>X</sub> proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the GPCR<sub>X</sub> proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the GPCR<sub>X</sub> proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding GPCR<sub>X</sub> proteins that contain changes in amino acid residues that are not essential for activity. Such GPCR<sub>X</sub> proteins differ in amino acid sequence from SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequences of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83; more preferably at least about 70% homologous to SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83; still more preferably at least about 80% homologous to SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83; even more preferably at least about 90% homologous to SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83; and most preferably at least about 95% homologous to SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83.

An isolated nucleic acid molecule encoding an GPCR<sub>X</sub> protein homologous to the protein of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the GPCR<sub>X</sub> protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an GPCR<sub>X</sub> coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for GPCR<sub>X</sub> biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant GPCR<sub>X</sub> protein can be assayed for (i) the ability to form protein:protein interactions with other GPCR<sub>X</sub> proteins, other cell-surface proteins, or biologically-active portions thereof, (ii) complex formation between a mutant GPCR<sub>X</sub> protein and an GPCR<sub>X</sub> ligand; or (iii) the ability of a mutant GPCR<sub>X</sub> protein to bind to an  
5 intracellular target protein or biologically-active portion thereof; (e.g. avidin proteins).

In yet another embodiment, a mutant GPCR<sub>X</sub> protein can be assayed for the ability to regulate a specific biological function (e.g., regulation of insulin release).

### Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules  
10 that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (e.g., complementary to the coding strand of a double-stranded cDNA  
15 molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire GPCR<sub>X</sub> coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an GPCR<sub>X</sub> protein of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36,  
20 38, 40, 42, 81, and 83, or antisense nucleic acids complementary to an GPCR<sub>X</sub> nucleic acid sequence of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an GPCR<sub>X</sub> protein. The term  
25 "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the GPCR<sub>X</sub> protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as  
30 5' and 3' untranslated regions).

Given the coding strand sequences encoding the GPCR<sub>X</sub> protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary



to the entire coding region of GPCR<sub>X</sub> mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of GPCR<sub>X</sub> mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of GPCR<sub>X</sub> mRNA. An antisense oligonucleotide can be, for example,  
5 about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or  
10 to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-  
15 2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopenentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil,  
20 2-methylthio-N6-isopenentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)<sub>w</sub>, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a  
25 nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or  
30 genomic DNA encoding an GPCR<sub>X</sub> protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense

nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other. See, e.g., Gaultier, *et al.*, 1987. *Nucl. Acids Res.* 15: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, e.g., Inoue, *et al.* 1987. *Nucl. Acids Res.* 15: 6131-6148) or a chimeric RNA-DNA analogue (see, e.g., Inoue, *et al.*, 1987. *FEBS Lett.* 215: 327-330).

#### Ribozymes and PNA Moieties

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave GPCR<sub>X</sub> mRNA transcripts to thereby inhibit translation of GPCR<sub>X</sub> mRNA. A ribozyme having specificity for an GPCR<sub>X</sub>-encoding nucleic acid can be designed based upon the nucleotide sequence of an GPCR<sub>X</sub> cDNA disclosed herein (i.e., SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an GPCR<sub>X</sub>-encoding

mRNA. See, e.g., U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* GPCR<sub>X</sub> mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, GPCR<sub>X</sub> gene expression can be inhibited by targeting nucleotide  
5 sequences complementary to the regulatory region of the GPCR<sub>X</sub> nucleic acid (e.g., the GPCR<sub>X</sub> promoter and/or enhancers) to form triple helical structures that prevent transcription of the GPCR<sub>X</sub> gene in target cells. See, e.g., Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the GPCR<sub>X</sub> nucleic acids can be modified at the base moiety,  
10 sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. See, e.g., Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (e.g., DNA mimics) in which the deoxyribose phosphate backbone is replaced by  
15 a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

20 PNAs of GPCR<sub>X</sub> can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of GPCR<sub>X</sub> can also be used, for example, in the analysis of single base pair mutations in a gene (e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in  
25 combination with other enzymes, e.g., S<sub>1</sub> nucleases (see, Hyrup, *et al.*, 1996. *supra*); or as probes or primers for DNA sequence and hybridization (see, Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNAs of GPCR<sub>X</sub> can be modified, e.g., to enhance their  
30 stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of GPCR<sub>X</sub> can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (e.g., RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA

chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see*, Hyrup, et al., 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, et al., 1996. *supra* and Finn, et al., 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.*, Mag, et al., 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.*, Finn, et al., 1996. *supra*. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.*, Petersen, et al., 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.*, Letsinger, et al., 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; Lemaitre, et al., 1987. *Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g.*, Krol, et al., 1988. *BioTechniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon, 1988. *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

### GPCRX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of GPCR<sub>X</sub> polypeptides whose sequences are provided in SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83 while still encoding a protein that maintains its GPCR<sub>X</sub> activities and physiological functions, or a functional fragment thereof.

In general, an GPCR<sub>X</sub> variant that preserves GPCR<sub>X</sub>-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or

residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

5           One aspect of the invention pertains to isolated GPCR<sub>X</sub> proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-GPCR<sub>X</sub> antibodies. In one embodiment, native GPCR<sub>X</sub> proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another  
10           embodiment, GPCR<sub>X</sub> proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an GPCR<sub>X</sub> protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

          An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue  
15           source from which the GPCR<sub>X</sub> protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of GPCR<sub>X</sub> proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes  
20           preparations of GPCR<sub>X</sub> proteins having less than about 30% (by dry weight) of non-GPCR<sub>X</sub> proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-GPCR<sub>X</sub> proteins, still more preferably less than about 10% of non-GPCR<sub>X</sub> proteins, and most preferably less than about 5% of non-GPCR<sub>X</sub> proteins. When the GPCR<sub>X</sub> protein or biologically-active portion thereof is recombinantly-produced, it is also preferably  
25           substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the GPCR<sub>X</sub> protein preparation.

          The language "substantially free of chemical precursors or other chemicals" includes preparations of GPCR<sub>X</sub> proteins in which the protein is separated from chemical precursors or  
30           other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of GPCR<sub>X</sub> proteins having less than about 30% (by dry weight) of chemical precursors or non-GPCR<sub>X</sub> chemicals, more preferably less than about 20% chemical precursors or non-GPCR<sub>X</sub> chemicals, still more preferably less than about 10% chemical precursors or

non-GPCRX chemicals, and most preferably less than about 5% chemical precursors or non-GPCRX chemicals.

Biologically-active portions of GPCRX proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the GPCRX proteins (*e.g.*, the amino acid sequence shown in SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83) that include fewer amino acids than the full-length GPCRX proteins, and exhibit at least one activity of an GPCRX protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the GPCRX protein. A biologically-active portion of an GPCRX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native GPCRX protein.

In an embodiment, the GPCRX protein has an amino acid sequence shown in SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83. In other embodiments, the GPCRX protein is substantially homologous to SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, and retains the functional activity of the protein of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the GPCRX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, and retains the functional activity of the GPCRX proteins of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83.

#### **Determining Homology Between Two or More Sequences**

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that

position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See*, Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

25

### Chimeric and Fusion Proteins

The invention also provides GPCR<sub>X</sub> chimeric or fusion proteins. As used herein, an GPCR<sub>X</sub> "chimeric protein" or "fusion protein" comprises an GPCR<sub>X</sub> polypeptide operatively-linked to a non-GPCR<sub>X</sub> polypeptide. An "GPCR<sub>X</sub> polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an GPCR<sub>X</sub> protein (SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83), whereas a "non-GPCR<sub>X</sub> polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the GPCR<sub>X</sub> protein, *e.g.*, a protein that is different from the GPCR<sub>X</sub> protein and that is derived from the same or a

different organism. Within an GPCR<sub>X</sub> fusion protein the GPCR<sub>X</sub> polypeptide can correspond to all or a portion of an GPCR<sub>X</sub> protein. In one embodiment, an GPCR<sub>X</sub> fusion protein comprises at least one biologically-active portion of an GPCR<sub>X</sub> protein. In another embodiment, an GPCR<sub>X</sub> fusion protein comprises at least two biologically-active portions of an GPCR<sub>X</sub> protein. In yet another embodiment, an GPCR<sub>X</sub> fusion protein comprises at least three biologically-active portions of an GPCR<sub>X</sub> protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the GPCR<sub>X</sub> polypeptide and the non-GPCR<sub>X</sub> polypeptide are fused in-frame with one another. The non-GPCR<sub>X</sub> polypeptide can be fused to the N-terminus or C-terminus of the GPCR<sub>X</sub> polypeptide.

In one embodiment, the fusion protein is a GST-GPCR<sub>X</sub> fusion protein in which the GPCR<sub>X</sub> sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant GPCR<sub>X</sub> polypeptides.

In another embodiment, the fusion protein is an GPCR<sub>X</sub> protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of GPCR<sub>X</sub> can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an GPCR<sub>X</sub>-immunoglobulin fusion protein in which the GPCR<sub>X</sub> sequences are fused to sequences derived from a member of the immunoglobulin protein family. The GPCR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an GPCR<sub>X</sub> ligand and an GPCR<sub>X</sub> protein on the surface of a cell, to thereby suppress GPCR<sub>X</sub>-mediated signal transduction *in vivo*. The GPCR<sub>X</sub>-immunoglobulin fusion proteins can be used to affect the bioavailability of an GPCR<sub>X</sub> cognate ligand. Inhibition of the GPCR<sub>X</sub> ligand/GPCR<sub>X</sub> interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (e.g. promoting or inhibiting) cell survival. Moreover, the GPCR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-GPCR<sub>X</sub> antibodies in a subject, to purify GPCR<sub>X</sub> ligands, and in screening assays to identify molecules that inhibit the interaction of GPCR<sub>X</sub> with an GPCR<sub>X</sub> ligand.

An GPCR<sub>X</sub> chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, e.g., by employing blunt-ended or stagger-ended termini for ligation, restriction



enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g., Ausubel, et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992*). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g., a GST polypeptide*). An GPCR<sub>X</sub>-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the GPCR<sub>X</sub> protein.

#### GPCR<sub>X</sub> Agonists and Antagonists

The invention also pertains to variants of the GPCR<sub>X</sub> proteins that function as either GPCR<sub>X</sub> agonists (*i.e., mimetics*) or as GPCR<sub>X</sub> antagonists. Variants of the GPCR<sub>X</sub> protein can be generated by mutagenesis (*e.g., discrete point mutation or truncation of the GPCR<sub>X</sub> protein*). An agonist of the GPCR<sub>X</sub> protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the GPCR<sub>X</sub> protein. An antagonist of the GPCR<sub>X</sub> protein can inhibit one or more of the activities of the naturally occurring form of the GPCR<sub>X</sub> protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the GPCR<sub>X</sub> protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the GPCR<sub>X</sub> proteins.

Variants of the GPCR<sub>X</sub> proteins that function as either GPCR<sub>X</sub> agonists (*i.e., mimetics*) or as GPCR<sub>X</sub> antagonists can be identified by screening combinatorial libraries of mutants (*e.g., truncation mutants*) of the GPCR<sub>X</sub> proteins for GPCR<sub>X</sub> protein agonist or antagonist activity. In one embodiment, a variegated library of GPCR<sub>X</sub> variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of GPCR<sub>X</sub> variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential GPCR<sub>X</sub> sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g., for phage display*) containing the set of

GPCRX sequences therein. There are a variety of methods which can be used to produce libraries of potential GPCR<sub>X</sub> variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential GPCR<sub>X</sub> sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. See, e.g., Narang, 1983. *Tetrahedron* 39: 3; Itakura, et al., 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, et al., 1984. *Science* 198: 1056; Ike, et al., 1983. *Nucl. Acids Res.* 11: 477.

### Polypeptide Libraries

In addition, libraries of fragments of the GPCR<sub>X</sub> protein coding sequences can be used to generate a variegated population of GPCR<sub>X</sub> fragments for screening and subsequent selection of variants of an GPCR<sub>X</sub> protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an GPCR<sub>X</sub> coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S<sub>1</sub> nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the GPCR<sub>X</sub> proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of GPCR<sub>X</sub> proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify GPCR<sub>X</sub> variants.

See, e.g., Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, et al., 1993. *Protein Engineering* 6:327-331.

### Anti-GPCR<sub>X</sub> Antibodies

The invention encompasses antibodies and antibody fragments, such as F<sub>ab</sub> or (F<sub>ab</sub>)<sub>2</sub>,  
5 that bind immunospecifically to any of the GPCR<sub>X</sub> polypeptides of said invention.

An isolated GPCR<sub>X</sub> protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind to GPCR<sub>X</sub> polypeptides using standard techniques for polyclonal and monoclonal antibody preparation. The full-length GPCR<sub>X</sub> proteins can be used or, alternatively, the invention provides antigenic peptide fragments of  
10 GPCR<sub>X</sub> proteins for use as immunogens. The antigenic GPCR<sub>X</sub> peptides comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83 and encompasses an epitope of GPCR<sub>X</sub> such that an antibody raised against the peptide forms a specific immune complex with GPCR<sub>X</sub>. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30  
15 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of GPCR<sub>X</sub> that is located on the surface of the protein (e.g., a  
20 hydrophilic region). As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation (see, e.g., Hopp and Woods, 1981. *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle, 1982. *J. Mol. Biol.* 157: 105-142, each incorporated herein  
25 by reference in their entirety).

As disclosed herein, GPCR<sub>X</sub> protein sequences of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers  
30 to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically-binds (immunoreacts with) an antigen, such as GPCR<sub>X</sub>. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F<sub>ab</sub> and F<sub>(ab)</sub><sub>2</sub> fragments, and an F<sub>ab</sub>

expression library. In a specific embodiment, antibodies to human GPCR<sub>X</sub> proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an GPCR<sub>X</sub> protein sequence of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly-expressed GPCR<sub>X</sub> protein or a chemically-synthesized GPCR<sub>X</sub> polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against GPCR<sub>X</sub> can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of GPCR<sub>X</sub>. A monoclonal antibody composition thus typically displays a single binding affinity for a particular GPCR<sub>X</sub> protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular GPCR<sub>X</sub> protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (*see, e.g.*, Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*see, e.g.*, Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the invention and may be produced by using human hybridomas (*see, e.g.*, Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an GPCR<sub>X</sub> protein (*see, e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F<sub>ab</sub> expression libraries (*see, e.g.*, Huse, *et al.*, 1989. *Science* 246: 1275-1281) to allow rapid and effective identification of

5 monoclonal F<sub>ab</sub> fragments with the desired specificity for an GPCR<sub>X</sub> protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. *See, e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to an GPCR<sub>X</sub> protein may be produced by techniques known in the art including, but not limited to: (i) an F<sub>(ab)<sub>2</sub></sub> fragment produced by pepsin digestion of an

10 antibody molecule; (ii) an F<sub>ab</sub> fragment generated by reducing the disulfide bridges of an F<sub>(ab)<sub>2</sub></sub> fragment; (iii) an F<sub>ab</sub> fragment generated by the treatment of the antibody molecule with papain and a reducing agent; and (iv) F<sub>v</sub> fragments.

Additionally, recombinant anti-GPCR<sub>X</sub> antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made

15 using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International

20 Publication No. WO 86/01533; U.S. Patent No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314 :446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.*

25 80: 1553-1559; Morrison(1985) *Science* 229:1202-1207; Oi, *et al.* (1986) *BioTechniques* 4:214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired

30 specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an GPCR<sub>X</sub> protein is facilitated by generation of hybridomas that bind to the fragment of an GPCR<sub>X</sub> protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an

GPCRX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-GPCR<sub>X</sub> antibodies may be used in methods known within the art relating to the localization and/or quantitation of an GPCR<sub>X</sub> protein (*e.g.*, for use in measuring levels of the GPCR<sub>X</sub> protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for GPCR<sub>X</sub> proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-GPCR<sub>X</sub> antibody (*e.g.*, monoclonal antibody) can be used to isolate an GPCR<sub>X</sub> polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-GPCR<sub>X</sub> antibody can facilitate the purification of natural GPCR<sub>X</sub> polypeptide from cells and of recombinantly-produced GPCR<sub>X</sub> polypeptide expressed in host cells. Moreover, an anti-GPCR<sub>X</sub> antibody can be used to detect GPCR<sub>X</sub> protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the GPCR<sub>X</sub> protein. Anti-GPCR<sub>X</sub> antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

### **GPCRX Recombinant Expression Vectors and Host Cells**

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an GPCR<sub>X</sub> protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a

"plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a  
5 bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general,  
10 expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

15 The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is  
20 intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and other  
25 expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*,  
30 tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including

fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., GPCR proteins, mutant forms of GPCR proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of GPCR proteins in prokaryotic or eukaryotic cells. For example, GPCR proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, e.g., Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (see, e.g.,



Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the GPCR<sub>X</sub> expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1  
5 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, GPCR<sub>X</sub> can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*,  
10 SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO*  
15 *J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*. 2nd ed., Cold Spring  
20 Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific  
25 promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament  
30 promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also

encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to GPCR<sub>X</sub> mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, GPCR<sub>X</sub> protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring

Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding GPCR<sub>X</sub> or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) GPCR<sub>X</sub> protein. Accordingly, the invention further provides methods for producing GPCR<sub>X</sub> protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding GPCR<sub>X</sub> protein has been introduced) in a suitable medium such that GPCR<sub>X</sub> protein is produced. In another embodiment, the method further comprises isolating GPCR<sub>X</sub> protein from the medium or the host cell.

## **Transgenic GPCR<sub>X</sub> Animals**

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which GPCR<sub>X</sub> protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous GPCR<sub>X</sub> sequences have been introduced into their genome or homologous recombinant animals in which endogenous GPCR<sub>X</sub> sequences have been altered. Such animals are useful for studying the function and/or activity of GPCR<sub>X</sub> protein and for identifying and/or evaluating modulators of GPCR<sub>X</sub> protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature

animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous GPCR<sub>X</sub> gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing GPCR<sub>X</sub>-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human GPCR<sub>X</sub> cDNA sequences of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human GPCR<sub>X</sub> gene, such as a mouse GPCR<sub>X</sub> gene, can be isolated based on hybridization to the human GPCR<sub>X</sub> cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the GPCR<sub>X</sub> transgene to direct expression of GPCR<sub>X</sub> protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: *MANIPULATING THE MOUSE EMBRYO*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the GPCR<sub>X</sub> transgene in its genome and/or expression of GPCR<sub>X</sub> mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding GPCR<sub>X</sub> protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an GPCR<sub>X</sub> gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the GPCR<sub>X</sub> gene. The GPCR<sub>X</sub> gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82), but more preferably, is a non-human homologue of a human GPCR<sub>X</sub> gene. For example, a mouse homologue of human GPCR<sub>X</sub> gene of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and

82 can be used to construct a homologous recombination vector suitable for altering an endogenous GPCR<sub>X</sub> gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous GPCR<sub>X</sub> gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous GPCR<sub>X</sub> gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous GPCR<sub>X</sub> protein). In the homologous recombination vector, the altered portion of the GPCR<sub>X</sub> gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the GPCR<sub>X</sub> gene to allow for homologous recombination to occur between the exogenous GPCR<sub>X</sub> gene carried by the vector and an endogenous GPCR<sub>X</sub> gene in an embryonic stem cell. The additional flanking GPCR<sub>X</sub> nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.*, Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced GPCR<sub>X</sub> gene has homologously-recombined with the endogenous GPCR<sub>X</sub> gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: *TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH*, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of

*Saccharomyces cerevisiae*. See, O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by  
5 mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.*, a somatic cell) from the transgenic animal can be isolated and induced to exit the  
10 growth cycle and enter G<sub>0</sub> phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell  
15 (*e.g.*, the somatic cell) is isolated.

#### Pharmaceutical Compositions

The GPCR<sub>X</sub> nucleic acid molecules, GPCR<sub>X</sub> proteins, and anti-GPCR<sub>X</sub> antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable  
20 for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most  
25 recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known  
30 in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, 5 intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, 10 and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous 15 solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under 20 the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by 25 the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the 30 composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an GPCR<sub>X</sub> protein or anti-GPCR<sub>X</sub> antibody) in the required amount in an appropriate solvent

with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.



In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

## Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express GPCR<sub>X</sub> protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect GPCR<sub>X</sub> mRNA (*e.g.*, in a biological sample) or a genetic lesion in an GPCR<sub>X</sub> gene, and to modulate GPCR<sub>X</sub> activity, as described further, below. In addition, the GPCR<sub>X</sub> proteins can be used to screen drugs or compounds that modulate the GPCR<sub>X</sub> protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of GPCR<sub>X</sub> protein or production of GPCR<sub>X</sub> protein forms that have decreased or aberrant activity compared to GPCR<sub>X</sub> wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease (possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-GPCR<sub>X</sub> antibodies of the invention can be used to detect and isolate GPCR<sub>X</sub> proteins and modulate GPCR<sub>X</sub> activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

## Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to GPCR<sub>X</sub> proteins or have a stimulatory or inhibitory effect on, *e.g.*, GPCR<sub>X</sub> protein expression or GPCR<sub>X</sub> protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an GPCR<sub>X</sub> protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries,

while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g., Lam, 1997. Anticancer Drug Design 12: 145.*

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.,* nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.,* Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of GPCR<sub>X</sub> protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an GPCR<sub>X</sub> protein determined. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the GPCR<sub>X</sub> protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the GPCR<sub>X</sub> protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with <sup>125</sup>I, <sup>35</sup>S, <sup>14</sup>C, or <sup>3</sup>H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the

assay comprises contacting a cell which expresses a membrane-bound form of GPCR<sub>X</sub> protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds GPCR<sub>X</sub> to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an GPCR<sub>X</sub> protein comprises determining the ability of the test compound to preferentially bind to GPCR<sub>X</sub> protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of GPCR<sub>X</sub> protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the GPCR<sub>X</sub> protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCR<sub>X</sub> or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the GPCR<sub>X</sub> protein to bind to or interact with an GPCR<sub>X</sub> target molecule. As used herein, a "target molecule" is a molecule with which an GPCR<sub>X</sub> protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an GPCR<sub>X</sub> interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An GPCR<sub>X</sub> target molecule can be a non-GPCR<sub>X</sub> molecule or an GPCR<sub>X</sub> protein or polypeptide of the invention. In one embodiment, an GPCR<sub>X</sub> target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound GPCR<sub>X</sub> molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with GPCR<sub>X</sub>.

Determining the ability of the GPCR<sub>X</sub> protein to bind to or interact with an GPCR<sub>X</sub> target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the GPCR<sub>X</sub> protein to bind to or interact with an GPCR<sub>X</sub> target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca<sup>2+</sup>, diacylglycerol, IP<sub>3</sub>, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an GPCR<sub>X</sub>-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*,

luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an GPCR<sub>X</sub> protein or biologically-active portion thereof with a test compound and  
5 determining the ability of the test compound to bind to the GPCR<sub>X</sub> protein or biologically-active portion thereof. Binding of the test compound to the GPCR<sub>X</sub> protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the GPCR<sub>X</sub> protein or biologically-active portion thereof with a known compound which binds GPCR<sub>X</sub> to form an assay mixture, contacting the assay mixture with a test  
10 compound, and determining the ability of the test compound to interact with an GPCR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an GPCR<sub>X</sub> protein comprises determining the ability of the test compound to preferentially bind to GPCR<sub>X</sub> or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting  
15 GPCR<sub>X</sub> protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of the GPCR<sub>X</sub> protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of GPCR<sub>X</sub> can be accomplished, for example, by determining the ability of the GPCR<sub>X</sub> protein to bind to an GPCR<sub>X</sub> target molecule by one of  
20 the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of GPCR<sub>X</sub> protein can be accomplished by determining the ability of the GPCR<sub>X</sub> protein further modulate an GPCR<sub>X</sub> target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the GPCR<sub>X</sub>  
25 protein or biologically-active portion thereof with a known compound which binds GPCR<sub>X</sub> protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an GPCR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an GPCR<sub>X</sub> protein comprises  
30 determining the ability of the GPCR<sub>X</sub> protein to preferentially bind to or modulate the activity of an GPCR<sub>X</sub> target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of GPCR<sub>X</sub> protein. In the case of cell-free assays comprising the membrane-bound form of GPCR<sub>X</sub> protein, it may be desirable to utilize a solubilizing agent

such that the membrane-bound form of GPCR<sub>X</sub> protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>,

- 5 Isotridecypoly(ethylene glycol ether)<sub>n</sub>, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either GPCR<sub>X</sub> protein or its target molecule to facilitate separation of  
10 complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to GPCR<sub>X</sub> protein, or interaction of GPCR<sub>X</sub> protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a  
15 fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-GPCR<sub>X</sub> fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or GPCR<sub>X</sub> protein, and the mixture  
20 is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of GPCR<sub>X</sub>  
25 protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the GPCR<sub>X</sub> protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated GPCR<sub>X</sub> protein or target molecules can be prepared from biotin-NHS  
30 (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with GPCR<sub>X</sub> protein or target molecules, but which do not interfere with binding of the GPCR<sub>X</sub> protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or GPCR<sub>X</sub> protein

trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the GPCR<sub>X</sub> protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the GPCR<sub>X</sub> protein or target molecule.

In another embodiment, modulators of GPCR<sub>X</sub> protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of GPCR<sub>X</sub> mRNA or protein in the cell is determined. The level of expression of GPCR<sub>X</sub> mRNA or protein in the presence of the candidate compound is compared to the level of expression of GPCR<sub>X</sub> mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of GPCR<sub>X</sub> mRNA or protein expression based upon this comparison. For example, when expression of GPCR<sub>X</sub> mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of GPCR<sub>X</sub> mRNA or protein expression. Alternatively, when expression of GPCR<sub>X</sub> mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of GPCR<sub>X</sub> mRNA or protein expression. The level of GPCR<sub>X</sub> mRNA or protein expression in the cells can be determined by methods described herein for detecting GPCR<sub>X</sub> mRNA or protein.

In yet another aspect of the invention, the GPCR<sub>X</sub> proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with GPCR<sub>X</sub> ("GPCR<sub>X</sub>-binding proteins" or "GPCR<sub>X</sub>-bp") and modulate GPCR<sub>X</sub> activity. Such GPCR<sub>X</sub>-binding proteins are also likely to be involved in the propagation of signals by the GPCR<sub>X</sub> proteins as, for example, upstream or downstream elements of the GPCR<sub>X</sub> pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for GPCR<sub>X</sub> is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to

interact, *in vivo*, forming an GPCR<sub>X</sub>-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with GPCR<sub>X</sub>.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

### Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

### Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the GPCR<sub>X</sub> sequences, SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, or fragments or derivatives thereof, can be used to map the location of the GPCR<sub>X</sub> genes, respectively, on a chromosome. The mapping of the GPCR<sub>X</sub> sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, GPCR<sub>X</sub> genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the GPCR<sub>X</sub> sequences. Computer analysis of the GPCR<sub>X</sub> sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the GPCR<sub>X</sub> sequences will yield an amplified fragment.



Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. See, e.g., D'Eustachio, *et al.*, 1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the GPCR sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, see, Verma, *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.*, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the GPCR<sub>X</sub> gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

#### Tissue Typing

The GPCR<sub>X</sub> sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the GPCR<sub>X</sub> sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The GPCR<sub>X</sub> sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding

regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

5 Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a  
10 noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

## 15 Predictive Medicine

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining GPCR<sub>X</sub> protein and/or  
20 nucleic acid expression as well as GPCR<sub>X</sub> activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant GPCR<sub>X</sub> expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders,  
25 Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with GPCR<sub>X</sub> protein, nucleic acid  
30 expression or activity. For example, mutations in an GPCR<sub>X</sub> gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with GPCR<sub>X</sub> protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining GPCR<sub>X</sub> protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics").

Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCR<sub>X</sub> in clinical trials.

These and other agents are described in further detail in the following sections.

### Diagnostic Assays

An exemplary method for detecting the presence or absence of GPCR<sub>X</sub> in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting GPCR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes GPCR<sub>X</sub> protein such that the presence of GPCR<sub>X</sub> is detected in the biological sample. An agent for detecting GPCR<sub>X</sub> mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to GPCR<sub>X</sub> mRNA or genomic DNA.

The nucleic acid probe can be, for example, a full-length GPCR<sub>X</sub> nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to GPCR<sub>X</sub> mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting GPCR<sub>X</sub> protein is an antibody capable of binding to GPCR<sub>X</sub> protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and

biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect GPCR<sub>X</sub> mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of GPCR<sub>X</sub> mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of GPCR<sub>X</sub> protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of GPCR<sub>X</sub> genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of GPCR<sub>X</sub> protein include introducing into a subject a labeled anti-GPCR<sub>X</sub> antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting GPCR<sub>X</sub> protein, mRNA, or genomic DNA, such that the presence of GPCR<sub>X</sub> protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of GPCR<sub>X</sub> protein, mRNA or genomic DNA in the control sample with the presence of GPCR<sub>X</sub> protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of GPCR<sub>X</sub> in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting GPCR<sub>X</sub> protein or mRNA in a biological sample; means for determining the amount of GPCR<sub>X</sub> in the sample; and means for comparing the amount of GPCR<sub>X</sub> in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect GPCR<sub>X</sub> protein or nucleic acid.

### Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant GPCR<sub>X</sub> expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with GPCR<sub>X</sub> protein, nucleic acid expression or activity.

Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant GPCR<sub>X</sub> expression or activity in which a test sample is obtained from a subject and GPCR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of GPCR<sub>X</sub> protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant GPCR<sub>X</sub> expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant GPCR<sub>X</sub> expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant GPCR<sub>X</sub> expression or activity in which a test sample is obtained and GPCR<sub>X</sub> protein or nucleic acid is detected (*e.g.*, wherein the presence of GPCR<sub>X</sub> protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant GPCR<sub>X</sub> expression or activity).

The methods of the invention can also be used to detect genetic lesions in an GPCR<sub>X</sub> gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an GPCR<sub>X</sub>-protein, or the misexpression of the GPCR<sub>X</sub> gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (i) a deletion of one or more nucleotides from an GPCR<sub>X</sub> gene; (ii) an addition of one or more nucleotides to an GPCR<sub>X</sub> gene; (iii) a substitution of one or more nucleotides of an GPCR<sub>X</sub> gene, (iv) a chromosomal rearrangement of an GPCR<sub>X</sub> gene; (v) an alteration in the level of a messenger RNA transcript of an GPCR<sub>X</sub> gene, (vi) aberrant modification of an GPCR<sub>X</sub> gene, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an GPCR<sub>X</sub> gene, (viii) a non-wild-type level of an GPCR<sub>X</sub> protein, (ix) allelic loss of an GPCR<sub>X</sub> gene, and (x) inappropriate post-translational

modification of an GPCR<sub>X</sub> protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an GPCR<sub>X</sub> gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran, *et al.*, 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the GPCR<sub>X</sub>-gene (*see*, Abravaya, *et al.*, 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an GPCR<sub>X</sub> gene under conditions such that hybridization and amplification of the GPCR<sub>X</sub> gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see*, Guatelli, *et al.*, 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification system (*see*, Kwoh, *et al.*, 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Q $\beta$  Replicase (*see*, Lizardi, *et al.*, 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an GPCR<sub>X</sub> gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.*, U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in GPCR<sub>X</sub> can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high-density arrays containing hundreds or thousands of oligonucleotide probes. *See, e.g., Cronin, et al., 1996. Human Mutation* 7: 244-255; Kozal, *et al.*, 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in GPCR<sub>X</sub> can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al., supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the GPCR<sub>X</sub> gene and detect mutations by comparing the sequence of the sample GPCR<sub>X</sub> with the corresponding wild-type (control) sequence.

Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g., Naeve, et al., 1995. Biotechniques* 19: 448), including sequencing by mass spectrometry (*see, e.g., PCT International Publication No. WO 94/16101; Cohen, et al., 1996. Adv. Chromatography* 36: 127-162; and Griffin, *et al., 1993. Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the GPCR<sub>X</sub> gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. *See, e.g., Myers, et al., 1985. Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type GPCR<sub>X</sub> sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S<sub>1</sub> nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide



and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g., Cotton, et al., 1988. Proc. Natl. Acad. Sci. USA 85: 4397; Saleeba, et al., 1992. Methods Enzymol. 217: 286-295.* In an embodiment, the control  
5 DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in GPCR<sub>X</sub> cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli*  
10 cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches. *See, e.g., Hsu, et al., 1994. Carcinogenesis 15: 1657-1662.* According to an exemplary embodiment, a probe based on an GPCR<sub>X</sub> sequence, *e.g.,* a wild-type GPCR<sub>X</sub> sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be  
15 detected from electrophoresis protocols or the like. *See, e.g., U.S. Patent No. 5,459,039.*

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in GPCR<sub>X</sub> genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g., Orita, et al., 1989. Proc. Natl. Acad. Sci. USA: 86: 2766; Cotton,*  
20 *1993. Mutat. Res. 285: 125-144; Hayashi, 1992. Genet. Anal. Tech. Appl. 9: 73-79.* Single-stranded DNA fragments of sample and control GPCR<sub>X</sub> nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with  
25 labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g., Keen, et al., 1991. Trends Genet. 7: 5.*

30 In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g., Myers, et al., 1985. Nature 313: 495.* When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich

DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g., Rosenbaum and Reissner, 1987. Biophys. Chem. 265: 12753.*

5 Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g., Saiki, et al., 1986. Nature 324: 163; Saiki, et al., 1989. Proc. Natl. Acad. Sci. USA 86: 6230.* Such allele specific oligonucleotides  
10 are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as  
15 primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g., Gibbs, et al., 1989. Nucl. Acids Res. 17: 2437-2448*) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g., Prossner, 1993. Tibtech. 11: 238*). In addition it may be desirable to introduce a novel  
20 restriction site in the region of the mutation to create cleavage-based detection. *See, e.g., Gasparini, et al., 1992. Mol. Cell Probes 6: 1.* It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g., Barany, 1991. Proc. Natl. Acad. Sci. USA 88: 189.* In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of  
25 a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.,* in clinical settings to diagnose  
30 patients exhibiting symptoms or family history of a disease or illness involving an GPCR<sub>X</sub> gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which GPCR<sub>X</sub> is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

### Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on GPCR<sub>X</sub> activity (e.g., GPCR<sub>X</sub> gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of GPCR<sub>X</sub> protein, expression of GPCR<sub>X</sub> nucleic acid, or mutation content of GPCR<sub>X</sub> genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See e.g., Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic

polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of GPCR<sub>X</sub> protein, expression of GPCR<sub>X</sub> nucleic acid, or mutation content of GPCR<sub>X</sub> genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an GPCR<sub>X</sub> modulator, such as a modulator identified by one of the exemplary screening assays described herein.

## Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GPCR<sub>X</sub> (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase GPCR<sub>X</sub> gene expression, protein levels, or upregulate GPCR<sub>X</sub> activity, can be monitored in clinical trails of subjects exhibiting decreased GPCR<sub>X</sub> gene expression, protein levels, or downregulated GPCR<sub>X</sub> activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease GPCR<sub>X</sub> gene expression, protein levels, or downregulate GPCR<sub>X</sub> activity, can be monitored in clinical trails of subjects exhibiting

increased GPCR<sub>X</sub> gene expression, protein levels, or upregulated GPCR<sub>X</sub> activity. In such clinical trials, the expression or activity of GPCR<sub>X</sub> and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

5 By way of example, and not of limitation, genes, including GPCR<sub>X</sub>, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates GPCR<sub>X</sub> activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of  
10 expression of GPCR<sub>X</sub> and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of GPCR<sub>X</sub> or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of  
15 the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the  
20 screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an GPCR<sub>X</sub> protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the GPCR<sub>X</sub> protein, mRNA, or genomic DNA in the  
25 post-administration samples; (v) comparing the level of expression or activity of the GPCR<sub>X</sub> protein, mRNA, or genomic DNA in the pre-administration sample with the GPCR<sub>X</sub> protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of GPCR<sub>X</sub> to higher levels  
30 than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of GPCR<sub>X</sub> to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

## Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant GPCR<sub>X</sub> expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Osteodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below.

### 15 Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be

utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an  
5 aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in*  
10 *situ* hybridization, and the like).

### Prophylactic Methods

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant GPCR<sub>X</sub> expression or activity, by administering to the  
15 subject an agent that modulates GPCR<sub>X</sub> expression or at least one GPCR<sub>X</sub> activity. Subjects at risk for a disease that is caused or contributed to by aberrant GPCR<sub>X</sub> expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the GPCR<sub>X</sub> aberrancy, such that a disease or disorder is  
20 prevented or, alternatively, delayed in its progression. Depending upon the type of GPCR<sub>X</sub> aberrancy, for example, an GPCR<sub>X</sub> agonist or GPCR<sub>X</sub> antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

25

### Therapeutic Methods

Another aspect of the invention pertains to methods of modulating GPCR<sub>X</sub> expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of GPCR<sub>X</sub> protein  
30 activity associated with the cell. An agent that modulates GPCR<sub>X</sub> protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an GPCR<sub>X</sub> protein, a peptide, an GPCR<sub>X</sub> peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more GPCR<sub>X</sub> protein activity. Examples of such stimulatory agents include active GPCR<sub>X</sub> protein and a nucleic acid molecule encoding

GPCRX that has been introduced into the cell. In another embodiment, the agent inhibits one or more GPCR<sub>X</sub> protein activity. Examples of such inhibitory agents include antisense GPCR<sub>X</sub> nucleic acid molecules and anti-GPCR<sub>X</sub> antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an GPCR<sub>X</sub> protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) GPCR<sub>X</sub> expression or activity. In another embodiment, the method involves administering an GPCR<sub>X</sub> protein or nucleic acid molecule as therapy to compensate for reduced or aberrant GPCR<sub>X</sub> expression or activity.

Stimulation of GPCR<sub>X</sub> activity is desirable in situations in which GPCR<sub>X</sub> is abnormally downregulated and/or in which increased GPCR<sub>X</sub> activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (e.g., cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (e.g., preclampsia).

#### Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

#### Prophylactic and Therapeutic Uses of the Compositions of the Invention

The GPCR<sub>X</sub> nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-



associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

5           As an example, a cDNA encoding the GPCR<sub>X</sub> protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's  
10   Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

          Both the novel nucleic acid encoding the GPCR<sub>X</sub> protein, and the GPCR<sub>X</sub> protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could  
15   be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

20

## EQUIVALENTS

          Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, which follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be  
25   made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims.

**WHAT IS CLAIMED IS:**

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83;
  - (b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;
  - (c) an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83; and
  - (d) a variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence.
2. The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence of a naturally-occurring allelic variant of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83.
3. The polypeptide of claim 2, wherein said allelic variant comprises an amino acid sequence that is the translation of a nucleic acid sequence differing by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82.

4. The polypeptide of claim 1, wherein the amino acid sequence of said variant comprises a conservative amino acid substitution.
5. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:
  - (a) a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83;
  - (b) a variant of a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of the amino acid residues from the amino acid sequence of said mature form;
  - (c) an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83;
  - (d) a variant of an amino acid sequence selected from the group consisting SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence;
  - (e) a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence chosen from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence; and
  - (f) a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e).
6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally-occurring allelic nucleic acid variant.

7. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule encodes a polypeptide comprising the amino acid sequence of a naturally-occurring polypeptide variant.
8. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82.
9. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:
  - (a) a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82;
  - (b) a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, provided that no more than 20% of the nucleotides differ from said nucleotide sequence;
  - (c) a nucleic acid fragment of (a); and
  - (d) a nucleic acid fragment of (b).
10. The nucleic acid molecule of claim 5, wherein said nucleic acid molecule hybridizes under stringent conditions to a nucleotide sequence chosen from the group consisting of SEQ ID NOS:1, 3, 4, 6, 8, 10, 12, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 80, and 82, or a complement of said nucleotide sequence.
11. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:
  - (a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 20% of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence;

- (b) an isolated second polynucleotide that is a complement of the first polynucleotide; and
  - (c) a nucleic acid fragment of (a) or (b).
- 12. A vector comprising the nucleic acid molecule of claim 11.
- 13. The vector of claim 12, further comprising a promoter operably-linked to said nucleic acid molecule.
- 14. A cell comprising the vector of claim 12.
- 15. An antibody that binds immunospecifically to the polypeptide of claim 1.
- 16. The antibody of claim 15, wherein said antibody is a monoclonal antibody.
- 17. The antibody of claim 15, wherein the antibody is a humanized antibody.
- 18. A method for determining the presence or amount of the polypeptide of claim 1 in a sample, the method comprising:
  - (a) providing the sample;
  - (b) contacting the sample with an antibody that binds immunospecifically to the polypeptide; and
  - (c) determining the presence or amount of antibody bound to said polypeptide, thereby determining the presence or amount of polypeptide in said sample.
- 19. A method for determining the presence or amount of the nucleic acid molecule of claim 5 in a sample, the method comprising:
  - (a) providing the sample;
  - (b) contacting the sample with a probe that binds to said nucleic acid molecule; and
  - (c) determining the presence or amount of the probe bound to said nucleic acid molecule, thereby determining the presence or amount of the nucleic acid molecule in said sample.

20. The method of claim 19 wherein presence or amount of the nucleic acid molecule is used as a marker for cell or tissue type.
21. The method of claim 20 wherein the cell or tissue type is cancerous.
22. A method of identifying an agent that binds to a polypeptide of claim 1, the method comprising:
  - (a) contacting said polypeptide with said agent; and
  - (b) determining whether said agent binds to said polypeptide.
23. The method of claim 22 wherein the agent is a cellular receptor or a downstream effector.
24. A method for identifying an agent that modulates the expression or activity of the polypeptide of claim 1, the method comprising:
  - (a) providing a cell expressing said polypeptide;
  - (b) contacting the cell with said agent, and
  - (c) determining whether the agent modulates expression or activity of said polypeptide,whereby an alteration in expression or activity of said peptide indicates said agent modulates expression or activity of said polypeptide.
25. A method for modulating the activity of the polypeptide of claim 1, the method comprising contacting a cell sample expressing the polypeptide of said claim with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.
26. A method of treating or preventing a GPCR<sub>X</sub>-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the polypeptide of claim 1 in an amount sufficient to treat or prevent said GPCR<sub>X</sub>-associated disorder in said subject.
27. The method of claim 26 wherein the disorder is selected from the group consisting of cardiomyopathy and atherosclerosis.

28. The method of claim 26 wherein the disorder is related to cell signal processing and metabolic pathway modulation.
29. The method of claim 26, wherein said subject is a human.
30. A method of treating or preventing a GPCR<sub>X</sub>-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the nucleic acid of claim 5 in an amount sufficient to treat or prevent said GPCR<sub>X</sub>-associated disorder in said subject.
31. The method of claim 30 wherein the disorder is selected from the group consisting of cardiomyopathy and atherosclerosis.
32. The method of claim 30 wherein the disorder is related to cell signal processing and metabolic pathway modulation.
33. The method of claim 30, wherein said subject is a human.
34. A method of treating or preventing a GPCR<sub>X</sub>-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired the antibody of claim 15 in an amount sufficient to treat or prevent said GPCR<sub>X</sub>-associated disorder in said subject.
35. The method of claim 34 wherein the disorder is diabetes.
36. The method of claim 34 wherein the disorder is related to cell signal processing and metabolic pathway modulation.
37. The method of claim 34, wherein the subject is a human.
38. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically-acceptable carrier.

39. A pharmaceutical composition comprising the nucleic acid molecule of claim 5 and a pharmaceutically-acceptable carrier.
40. A pharmaceutical composition comprising the antibody of claim 15 and a pharmaceutically-acceptable carrier.
41. A kit comprising in one or more containers, the pharmaceutical composition of claim 38.
42. A kit comprising in one or more containers, the pharmaceutical composition of claim 39.
43. A kit comprising in one or more containers, the pharmaceutical composition of claim 40.
44. A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 1 in a first mammalian subject, the method comprising:
- (a) measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and
  - (b) comparing the amount of said polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second mammalian subject known not to have, or not to be predisposed to, said disease;
- wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the presence of or predisposition to said disease.
45. The method of claim 44 wherein the predisposition is to cancers.



46. A method for determining the presence of or predisposition to a disease associated with altered levels of the nucleic acid molecule of claim 5 in a first mammalian subject, the method comprising:
- (a) measuring the amount of the nucleic acid in a sample from the first mammalian subject; and
  - (b) comparing the amount of said nucleic acid in the sample of step (a) to the amount of the nucleic acid present in a control sample from a second mammalian subject known not to have or not be predisposed to, the disease;
- wherein an alteration in the level of the nucleic acid in the first subject as compared to the control sample indicates the presence of or predisposition to the disease.
47. The method of claim 46 wherein the predisposition is to a cancer.
48. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a polypeptide in an amount that is sufficient to alleviate the pathological state, wherein the polypeptide is a polypeptide having an amino acid sequence at least 95% identical to a polypeptide comprising an amino acid sequence of at least one of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, or a biologically active fragment thereof.
49. A method of treating a pathological state in a mammal, the method comprising administering to the mammal the antibody of claim 15 in an amount sufficient to alleviate the pathological state.
50. A method for the screening of a candidate substance interacting with an olfactory receptor polypeptide selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, or fragments or variants thereof, comprises the following steps:

- a) providing a polypeptide selected from the group consisting of the sequences of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, or a peptide fragment or a variant thereof;
  - b) obtaining a candidate substance;
  - c) bringing into contact said polypeptide with said candidate substance;
  - and
  - d) detecting the complexes formed between said polypeptide and said candidate substance.
51. A method for the screening of ligand molecules interacting with an olfactory receptor polypeptide selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, wherein said method comprises:
- a) providing a recombinant eukaryotic host cell containing a nucleic acid encoding a polypeptide selected from the group consisting of the polypeptides comprising the amino acid sequences SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83;
  - b) preparing membrane extracts of said recombinant eukaryotic host cell;
  - c) bringing into contact the membrane extracts prepared at step b) with a selected ligand molecule; and
  - d) detecting the production level of second messengers metabolites.
52. A method for the screening of ligand molecules interacting with an olfactory receptor polypeptide selected from the group consisting of SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83, wherein said method comprises:
- a) providing an adenovirus containing a nucleic acid encoding a polypeptide selected from the group consisting of polypeptides comprising the amino acid sequences SEQ ID NOS:2, 5, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 81, and 83;
  - b) infecting an olfactory epithelium with said adenovirus;
  - c) bringing into contact the olfactory epithelium b) with a selected ligand molecule; and
  - d) detecting the increase of the response to said ligand molecule.